TdT Assay

should be 50 mM

pool	per tube		final concentration
25λ	2.5λ	1M K cacodylate, pH 7.0	100mM
5λ	0.5λ	50mM $CoCl_2$	1mM
10λ	1λ	1:100 dilution of 0.5M dTT	0.2mM dTT
70λ	7λ	poly d(A) ~~100~~ 50	~~6μM 3'(OH)~~ .014μM
50λ	5λ	5mM d(GTP (1:10 in label)	1mM d GTP
	9λ	H_2O (^3H)	
	1λ	enzyme, straight or diluted	

	TdT #1 (many very small vials)	TdT #2 (large screw top vial)
and 1 enzyme added	.01λ ⇒ "1-.01"	.01λ ⇒ "2-.01"
	.1λ ⇒ "1-.1"	.1λ ⇒ "2-.1"
	1λ ⇒ "1-1"	1λ ⇒ "2-1"

1. Reaction incubated at 37°C for one hour

```
          USER  1

SR  CH  12    1 TIMES
CR            1 TIMES
SCR =OFF
AUC =OFF
SF  =ON
CHLC=         1
PST =     10.00 MIN
CH 1       2.00 2 SIGMA %
             .0 LSR
             .0 BKG
            .00 2 SIGMA B
              0 LL
             39% UL
CH 2       2.00 2 SIGMA %
             .0 LSR
             .0 BKG
            .00 2 SIGMA B
             655 LL
             940 UL
```

POS	CH 1	2S%	CH 2	2S%	TIME
150 1-1	823.2	2.2	10.3	19.6	10.00
151 1-1	748.7	2.3	9.6	20.3	10.00
152 1-.01	711.8	2.3	11.2	18.8	10.00
153 2-1	542.2	2.7	13.7	17.0	10.00
154 2-1	924.0	2.0	11.5	18.6	10.00
	623.9	2.5	21.8	13.5	10.00

These calculations have many errors, see p. 10

$\rightarrow$ 2000 pg/ml = 2000 μg/l

true molarity of DNA A(mw) ≅ 347 g/mole poly(A)$_{100}$ (mw) = (347)(100)

$$\frac{(2.5\,\mu g \times 10^{-6}\,g/\mu g)(8 \times 10^{-3}\,ml)}{(100)(347\,g/mole)} = 3.60 \times 10^{-13}\,moles$$

(8 should be 7; 100 should be 50)

$$\frac{3.60 \times 10^{-13}\,moles}{25 \times 10^{-6}\,l} = 1.44 \times 10^{-8}\,M = .044\,\mu M$$

G's added

$$(1 \times 10^{-3}\,M/l)(25 \times 10^{-6}\,l)N = \#\,molecules\ of\ G$$

N = Avogadro's number

$$(1.44 \times 10^{-8}\,M/l)(25 \times 10^{-6}\,l)N = \#\,molecules\ of\ 3'OH\ ends$$

max possible incorporation of G

$$= \frac{(1 \times 10^{-3})(25 \times 10^{-6})N}{(1.44 \times 10^{-8})(25 \times 10^{-6})N} = \frac{9 \times 10^{-3}}{1.44 \times 10^{-8}} = 69{,}444\ G's\ per\ 3'\ end$$

data here uses 1-1-1 and 1-1-2

actual incorporation

$$\frac{800\ cpm}{(1360\ cpm)(50)} = .0118$$

(1360 cpm = pool dot; 50 = dil. of pool spot; 250)

$$(.0118)(69{,}444) = 819$$

If 2000 cpm

$$\frac{2000\ cpm}{(1360)(50)} = .0294$$

$$(.0294)(69{,}444) = 2042$$

$(\frac{1}{250})($

1-2-021 T=010.00 A=002094.0(1.5%) B=002556.8(1.5%) C=002600.1(1.5%) R=1.
1-2-022 T=010.00 A=000778.2(3.0%) B=000972.7(3.0%) C=001030.8(2.0%) R=1.
1-2-023 T=010.00 A=000151.4(7.0%) B=000187.7(5.0%) C=000214.1(5.0%) R=1.
1-2-024 T=010.00 A=000048.8(10%) B=000060.9(10%) C=000083.6(7.0%) R=1.2
1-2-025 T=010.00 A=000034.4(15%) B=000042.6(10%) C=000069.8(10%) R=1.2

1-2-026 T=010.00 A=000029.5(15%) B=000039.3(15%) C=000063.7(10%) R=1.3
1-2-027 T=010.00 A=000028.1(15%) B=000033.0(15%) C=000059.1(10%) R=1.17
1-2-028 T=010.00 A=000031.9(15%) B=000041.4(10%) C=000064.7(10%) R=1.32
1-2-029 T=010.00 A=000387.7(5.0%) B=000412.5(3.0%) B=000443.7(3.0%) R=1.25
1-2-030 T=010.00 A=000155.3(5.0%) B=000192.5(5.0%) C=000256.1(10%) R=1.20

2-01-2-031 T=010.00 A=000023.2(15%) B=000030.2(15%) C=000056.5(10%) R=1.39
2-01-1-032 T=010.00 A=000025.7(15%) B=000033.4(15%) C=000059.3(10%) R=1.56
2-01-2-033 T=010.00 A=000030.1(15%) B=000036.8(15%) C=000061.7(10%) R=1.20
2-02-1-034 T=010.00 A=000026.1(15%) B=000034.2(15%) C=000057.2(10%) R=1.39
2-02-2-035 T=010.00 A=000028.8(15%) B=000036.4(15%) C=000061.4(10%) R=1.38

2-03-1-036 T=010.00 A=000030.9(15%) B=000039.6(15%) C=000065.5(10%)
2-03-2-037 T=010.00 A=000112.5(7.0%) B=000140.9(7.0%) C=000167.7(5.0%)

2-L-2-038 T=010.00 A=000086.5(7.0%) B=000111.9(7.0%) C=000133.7(7.0%) R=1.
2-H-1-039 T=010.00 A=000026.0(15%) B=000033.6(15%) C=000058.9(10%) R=1.
2-H-2-040 T=010.00 A=000022.3(15%) B=000031.6(15%) C=000054.1(10%) R=1.4

3-01-1-041 T=010.00 A=000024.4(15%) B=000035.6(15%) C=000058.8(10%) R=1.458
3-01-2-042 T=010.00 A=000030.3(15%) B=000042.9(10%) C=000065.5(10%) R=1.400
3-02-1-043 T=010.00 A=000020.5(15%) B=000031.7(15%) C=000056.2(10%) R=1.550
3-02-2-044 T=010.00 A=000022.2(15%) B=000033.0(15%) C=000056.4(10%) R=1.500
C1-045 T=010.00 A=000025.4(15%) B=000034.7(15%) C=000059.1(10%) R=1.366

C-2-046 T=010.00 A=000026.5(15%) B=000039.8(15%) C=000064.4(10%) R=1.500
Pool-047 T=010.00 A=001352.4(2.0%) B=001717.6(2.0%) C=001738.5(2.0%) R=1.26
Pool-048 T=010.00 A=001362.6(2.0%) B=001718.0(2.0%) C=001733.7(2.0%) R=1.2

corrected for error on p. 2

Unit Calculation – Lamy's Way

$1mM\ dGTP = \frac{10}{1nM}/\lambda$

10λ of a 1:100 dilution of pool = 0.1λ spotted
spotted

this produced 1360 counts

~~13,600~~ 1,360 cpm/nmole

~~13,600 cpm/nmole~~

Assumed highest (+2) → $\frac{2000\ cpm}{\frac{~~13,600~~\ 1,360\ cpm}{nmole}} = \frac{1.5}{~~.15~~}$ nmole incorporated

$\frac{1.5}{~~.15~~}$ units/λ

→

$\frac{1360 counts}{.1\lambda}\ \frac{10nM}{\lambda}$

$1360\ \frac{CPM}{~~counts~~}\ \frac{\lambda}{10nM} = 1360\ \frac{cpm}{nM}$

PuC19 1µg/λ

SphI ≡ P-S

5λ PuC19 (= 5µg)
2λ SphI (= 10 units)
2.5λ 10X Low Salt buffer
15.5λ H_2O
25λ total

≡ λ-S .6µg/λ

1λ λ DNA (600µg/ml)
1λ SphI (= 5 units)
2.5λ 10X L. Salt buffer
20.5λ H_2O
25λ total

HindIII ≡ P-H

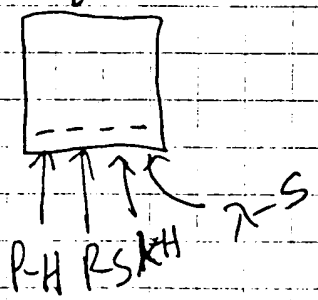
5λ PuC19 (= 5µg)
2λ HindIII (= 8 units)
2.5λ 10X "cure" buffer
15.5λ H_2O
25λ total

≡ λ-H

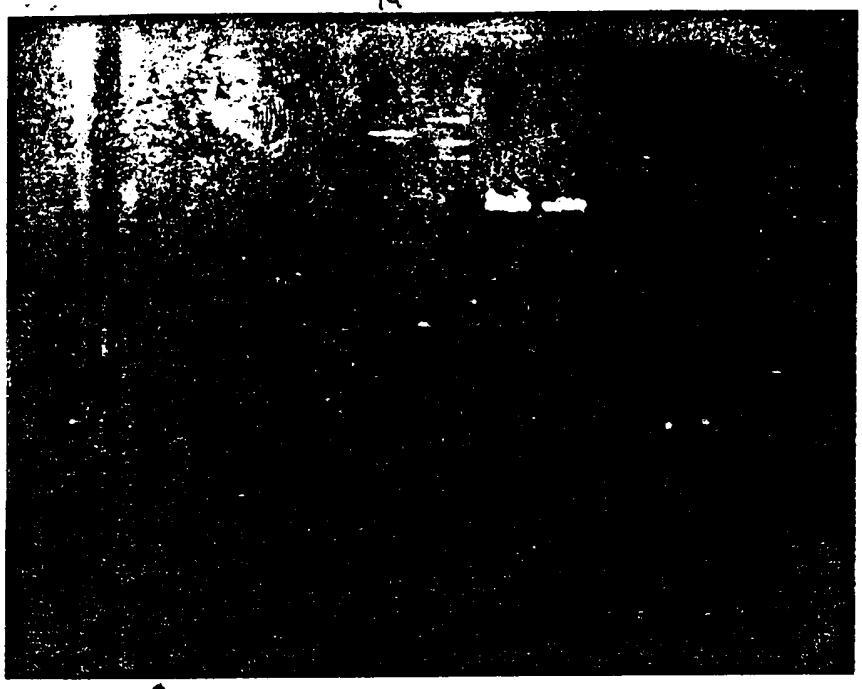
1λ λ DNA
1λ HindIII (= 4 units)
2.5λ 10X "cure" buffer
20.5λ H_2O
25λ total

37° at 10:15

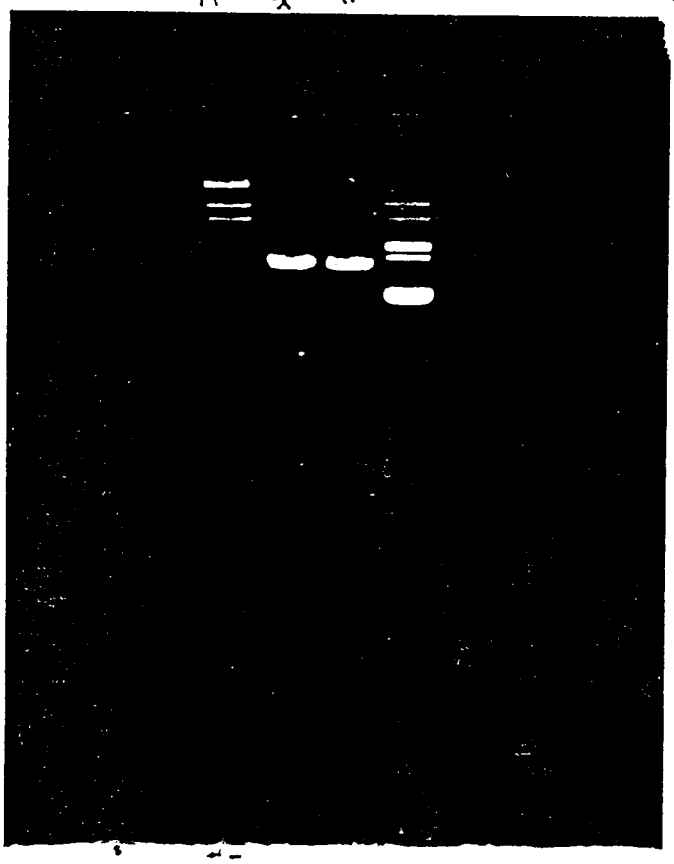
gel
TBE (diluted from 10X buffer)
.7% agarose



λ/SphI λ/HindIII pUC-SphI pUC-HindIII



λ/HindIII SphI HindIII pUC Alone



Phenol extraction & EtOH ppt of DNA

1. dilute DNA to 200λ w/ TE
2. Equilibrate Phenol w/ TE & adjust pH to 8.0 w/ 10N N
3. Add 200λ (equal volume) phenol
4. Spin & suck off phenol (bottom of 2 phases)
5. Spin again & again suck off phenol
6. add 500λ diethyl ether and let evaporate in hood (but mix first) to remove leftover phenol
7. ppt w/ 22λ 3M NH_4 acetate
   550λ of 95% EtOH
8. ppt @ -70°C, pellet 5' in μfuge, drain & wash ppt w/ cold 70% EtOH and vac dry

eluted in 15λ TE

TdT Assay using Rxn 19

~~Enzyme Dilution~~ ~~pool~~ (plan first 12 labsdays)

pool	per tube		
250λ	2.5λ	cacodylate	Pool = 9λ/tube
50λ	0.5λ	$CoCl_2$	
100λ	1λ	1:100 dilution of DTT	
500λ	5λ	dGTP (1:10 in label)	
450 dGTP sodium	1λ	enzyme, undilated	
	2λ	DNA (=.8μg) @ .27μg/1λ	
	13λ	H_2O	
	25λ total		

↳ this amt is roughly scaled up from the poly(A)100 (multiplied by 50 (=2×25) since this DNA is ds & about 25× heavier – but it does have 2 ends upon which to add)

2 trials each of pUC/HIII and pUC/SPH1
+ control of pool + H_2O

```
PROG  6        USER  1

SR  CH  12     1 TIMES
CR             1 TIMES
SCR =OFF
AGC =OFF
SF  =ON
CALC=          1
PST =     10.00 MIN
CH 1       2.00 2 SIGMA %
             .0 LSR
             .0 BKG
            .00 2 SIGMA B
              0 LL
            397 UL
CH 2       2.00 2 SIGMA %
             .0 LSR
             .0 BKG
            .00 2 SIGMA B
            655 LL
            940 UL
```

POS		CH 1	2S%	CH 2	2S%	TIME
139	HindIII-1	27.7	12.0	9.2	20.8	10.00
140	HindIII-2	29.0	11.7	9.2	20.8	10.00
141	SphI-1	32.8	11.0	7.5	23.0	10.00
142	SphI-2	35.2	10.6	9.5	20.5	10.00
143	Control	21.5	13.6	11.4	18.7	10.00
144	Pool	4575.3	.9	7.7	22.7	10.00

↳ 10 λ of 1:100 dilution

TdT Assay

9λ Pool
7λ poly(A) 100
1λ enzyme
8λ H_2O
25λ

as positive control

9λ Pool
1λ enzyme HindIII
4λ puc
11λ H_2O SphI
25λ

↳ 10:30 pm

Rxn stopped after 1/2 hour and ppt O/N in cold room

TdT Assay

pool	per tube		final concentration
100λ	25λ	cacodylate	100mM
20λ	0.5λ	$CoCl_2$	1mM
40λ	1λ	1:100 dilution of Oitt	0.2mM
280λ	7λ	poly(A)	~~2mM 3'OH~~ .04mM
200λ	5λ	d6TPCl(1:10 in label)	1mM d6TP
	8λ	H_2O	
	1λ	enzyme, straight & serially diluted	

3 batches of enzyme were tried at 1λ, .1λ, .01λ and .001λ each. Batch 1 was already sorted into small vials. Batch 2 was in the large tube w/ a cap. Vial 3 was uncapped and covered w/ parafilm.

"A-B-C" ⇒ A = batch # (1, 2 or 3)
B = quantity of enzyme used (1λ, .1λ, .01λ, or .001λ)
C = trial number (1 or 2, two trials of each possible used)

C1 = Control #1
C2 = Control #2
} pool w/o enzyme in same amt per tube (25λ − 1λ = 24λ)

pool1
pool2
} 10λ of a 1:100 dilution of the pool spotted on a filter and dried

not purified in large quantities

TdT Assay

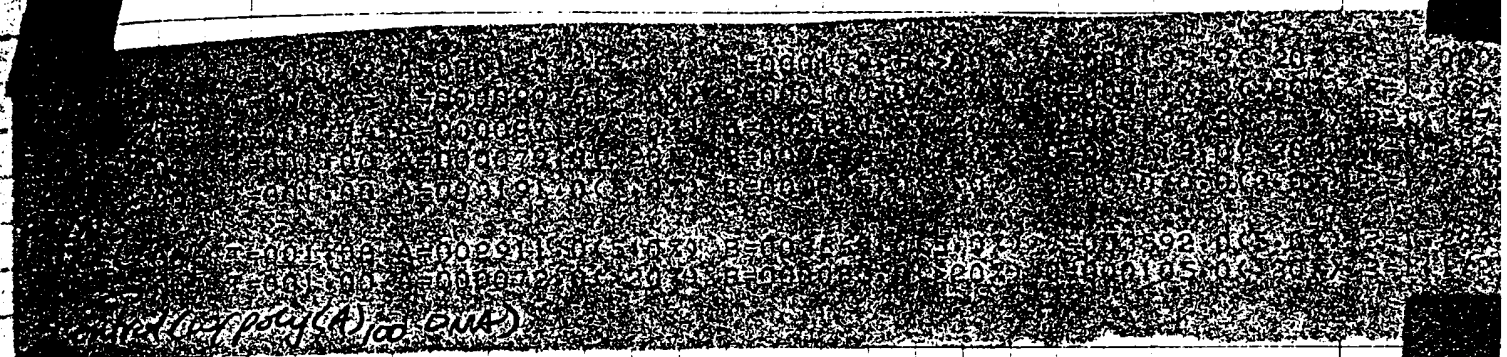
poly(A)	Puc/HIII	Puc/SphI	λ HIII
9λ pool	9λ pool	9λ pool	9λ pool
7λ poly(A) 100	2λ DNA (@.22 μg/λ)	2λ DNA	2λ DNA
1λ enzyme	5λ enzyme	5λ enzyme	5λ enzyme
17λ	16λ	16λ	16λ

2 → 30 pm ↳ 50' before stopping

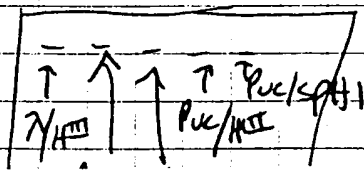
T=001.50 A=000138.6(15%) B=000144.6(15%) C=000166.6(15%) S=1.050
T=000.50 A=000184.0(>20%) B=000192.0(>20%) C=000222.0(>20%) S=1.000

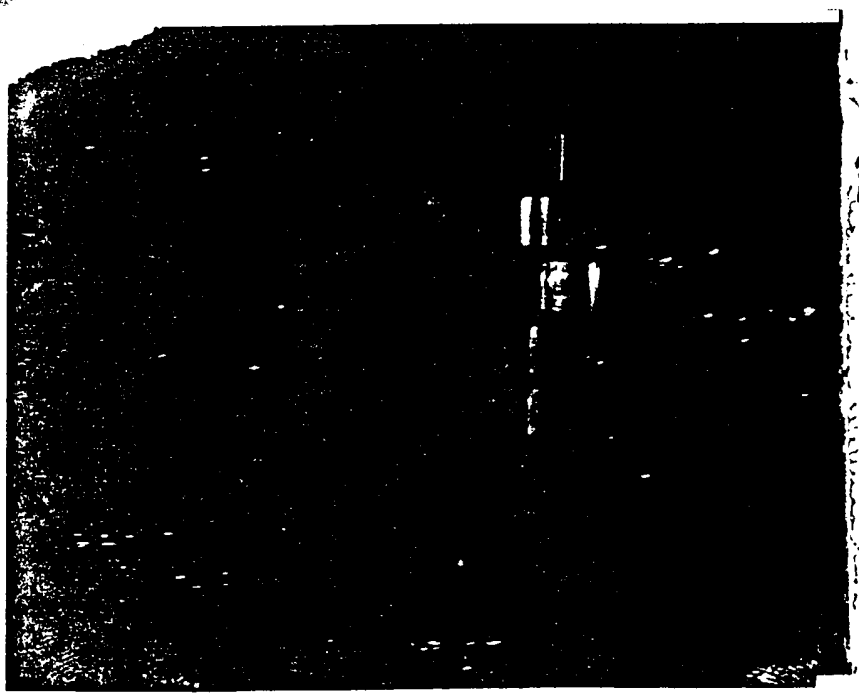
T=000.50 A=000162.0(>20%) B=000153.0(>20%) C=000202.0(>20%) S=1.275
T=000.50 A=000055.0(>20%) B=000076.0(>20%) C=000098.0(>20%) S=0.975

The above rxn used poly(A)$_{100}$ as a positive control (which worked ⇒ the problem is in the DNA and not the ~~[illegible]~~ terminal transferase)



Gel to find out if DNA present





λ/HIII
PUC
"PUC" → from unreliable source
PUC/HIII
PUC/SphI

⇒ DNA present

TdT Assay

pool	perfuse		final concentrat
125λ	2.5λ	1M KTcacodylate pH7.0	100mM KTcac
25λ	0.5λ	50mM $CoCl_2$	1mM $CoCl_2$
50λ	1λ	1:100 dilution of 0.5M dTT	0.2mM dTT
25λ	0.5λ	5mM dGTP	1mM dGTP
250λ	5λ	.087mM ³H-dGTP	.017mM d*GTP
	1λ	.8μg/λ DNA (=.8μg)	1.64×10⁻⁷M
	1λ	enzyme	11
	13.5λ	H_2O	dGTP / 3'ends = 6,000

HindIII digest of PvuI3

90λ PvuI3 DNA (1μg/λ ⇒ 90μg)
22λ HindIII (=88 units)
12.4 10× Core buffer 4:30pm
124.4λ total

Digest was checked after 5hrs, but then allowed to go O/N

Taurine Glycine CBRC ~8 units/kJ

30 Minutes

poly(A) 010 T=000.50 A=000168.0(>20%) B=000155.0(>20%) C=000202.0(20%) R=1.000
poly3 015 T=000.50 A=000144.0(>20%) B=000152.0(>20%) C=000182.0(>20%) S=1.000

after 4 hours

1. λ/EcoRI
2. PuC19 uncut
3. PuC13 uncut
4. 13/HindIII

TdT Assay

poly(A) 50	PuC13/HindIII	PuC13/HindIII + Poly(A)	Control
9λ pool	9λ pool	9λ pool	
7λ poly(A)	1 [illegible] DNA	1 [illegible] DNA	
1λ enzyme	1λ enzyme	7λ poly(A)	
8λ H_2O	14.5λ H_2O	1λ enzyme	
25λ	25λ	7.5λ H_2O	
		25λ	

↳ 30 min.

000.50 A=002976.0(7.0%) B=003714.9(5.0%) C=003770.0(5.0%) R=1.250
000.50 A=000148.0(>20%) B=000128.0(>20%) C=000152.0(>20%) R=1.900
000.50 A=004618.0(5.0%) B=005926.0(5.0%) C=005984.0(5.0%) R=1.296

#6's per 3' end of Puc13

Molarity of Puc13:

$$\frac{(1\times10^{-6}\ g)(1\ \text{mole})}{1.8\times10^{6}\ g} = 5.56\times10^{-13}\ \text{moles (in 1 } \mu\text{l of total DNA)}$$

~~$\frac{5.56\times10^{-13}\ \text{moles}}{.025\ \text{ml}} = 2.22\times10^{-11}$ mM~~

~~there are 2 3' ends per molecule $\Rightarrow (2)(2.22\times10^{-11}\ \text{mM}) = 4.44\times10^{-11}$ mM~~

(cf. Wu who recommends ~~60 pmole/ml $= 6\times10^{-11}$ mM~~)

$$\frac{5.56\times10^{-13}\ \text{moles}}{25\times10^{-6}\ l} = 2.22\times10^{-8}\ M = 2.22\times10^{-5}\ \text{mM}$$

remember 2 3' ends/molecule $\Rightarrow 4.44\times10^{-5}$ mM (3'

Molarity of dGTP

$4.5\ \mu l \rightarrow 25\ \mu l \Rightarrow \frac{4.5}{25} = .18$ dilution

$(.18)(50\ \text{mM}) = 9\ \text{mM}$

~~GTP / PolC3 = 9 nM / 2.22×10⁻⁵ nM = 405,405 G's/PolC3~~ (not possible → stop)

$\frac{GTP}{PolC3} = \frac{9\ nM}{4.44\times10^{-5}\ nM} = 202{,}703$ G/3'OH

fraction of counts incorporated ← background

$\frac{118-30}{(250\times4575)\ cpm} = \frac{88}{(250\times4575)} = 7.7\times10^{-5}$

.17 g washed pool → 4575 cpm in 25 ng pool

there are (250×4575 cpm)

actual incorporation:

$7.7\times10^{-5}\ (202{,}703) =$ **15 G/3' end**

#G's per 3' end on poly(A)

molarity of poly(A) = (see also p. 4)

~~(2.5×10⁻⁶ g)~~

$\frac{(2.5\times10^{-6}\ g)(7\times10^{-3}\ mol)}{(50)(347\ g/mole)} = 5.04\times10^{-11}$ mole

$\frac{5.04\times10^{-11}\ mole}{25\times10^{-6}\ l} = 2.02\times10^{-6}\ M$

$= 2.02\times10^{-3}\ mM$

max possible incorp.

$\frac{GTP}{PolC3} = \frac{9\ nM}{2.02\times10^{-3}\ mM} = 4{,}455$ G/3'OH

4/11

Notice that although $\frac{G}{3'end}$ is about the same for both PvC13/HIII and poly(A)50 the ratio of $\frac{GTP}{3'OH}$ is greater for PvC13 (⇒ equilibrium-mass action-effects should be stronger)

Since $\frac{GTP}{3'OH}$ for PvC13 is about 50X greater than the ratio for poly(A)50 and the amt of incorp is about the same, this suggests the PvC13 rxn is 50X less efficient than poly(A)50

(Obvious: The reason why there is relatively few counts depicted for PvC13/HIII is because the radioactivity of this is much less than that of the poly(A)50 as used in the same assay. To better differentiate background from incorporation in the PvC rxn, I should ↑ the amt of label in the pool-by-sens, 10X (sounds good).)

fraction actually incorporated

$$\frac{30000}{(250)(4575)} = .0026$$

actual incorp $(.0026)(4,455) = \boxed{12\ 6/3\ cpd}$

The molarity of ^3HdGTP in stock

data from spec sheet →

$$\frac{.013 \times 10^{-3} g}{(.25 \times 10^{-3} L)(595.29 g/mol)} = 8.7 \times 10^{-5} M = 87 \times .087 mM$$

pUC13/HIII	pUC13/HIII	Control
9λ pool	9λ pool	9λ pool
7λ poly(A)	1λ DNA	2λ DNA (pUC13/HIII)
1λ enzyme	1λ enzyme	1λ enzyme
8λ H_2O	14λ H_2O	8λ H_2O
25λ total	25λ total	20λ total

put in at 37° at 3:30pm
↳ allowed to go for 18 hours

[redacted]

poly(A)50 →

015 T=000.50 A=004418.0(5.0%) B=005312.0(5.0%) C=005360.0(5.0%) R=1.204

pUC13/(HIII) →

016 T=000.50 A=000178.0(>20%) B=000176.0(>20%) C=000800.0(>20%) R=1.000

control → (poly(A) DNA)

017 T=000.50 A=000052.0(>20%) B=000070.9(>20%) C=000104.0(>20%) R=1.000

18 HOURS A=50%

[redacted]

$PdCl_3/H^{III} \Rightarrow 226$ / 3' end

$pdyd(A)_{50} \Rightarrow 176$ / 3' end

dGTP, Na^+ salt

~~507.20~~ 609 g/mole

Pt drew has .025g

1ml 1M soution

TdT Assay

This assay uses 1/10 and 1/100 the molar concentration of PUC13/HIII as the previous series. This effectively ↑ the relative d6TP/3'OH ratio by a factor of 10. Additionally the label has been increased by 14-fold. (There should be more incorporation per 3' end, but there are fewer 3' ends, so this increase is not expected to appreciably alter the counts, relative to background.)

1. $\frac{1}{10}$ the previous concentration of DNA ⇒ 2.03×10^6 excess of d6TP over 3'

✓	1λ	of a 1:10 dilution of 1μg/1λ PUC13/HIII DNA	.1 μg = 4.44×10^{-6} mM
	1λ	TdT	3 units
✓	5λ	50mM d6TP	10mM
	14λ	^{3}H d6TP	.05mM
✓ Pool ≡ 54λ	2.5λ	1M KCacodylate	100mM
	0.5λ	5mM CoCl$_2$	1mM
	1λ	1:100 dilution of 0.5M diTT	02mM diTT
	25λ	total	

2. ~~1λ of a 1:100 dilution of~~ $\frac{1}{100}$ the previous concentration of DNA ⇒ 2.03×10^7 excess of d6TP over 3'

1λ of a 1:100 dilution of 1μg/1λ PUC13/HIII DNA — .01 μg = 4.44×10^{-7} mM

the rest is the same as the above assay

25λ

3. As before, 1λ of 1μg/1λ PUC13/HIII DNA

The rest is the same as above

4. Control, everything but no DNA or enzyme — 2.03×10^5 Co/3'end

1 λ of the 23λ total control rxn was spotted for use as a "pad spot"

Pool ⊖ ← signifies no dGTP
(enough for 100 25λ assays)

250λ cacodylate
50λ $CoCl_2$
99λ H_2O
1λ undiluted 0.5M diTT

2 pools were prepared - red label $CoCl_2$ added before diTT added (last) - turned brown - not used. When $CoCl_2$ was added last there was no brown ppt. This good stuff is in a green labelled tube and was used for the above rxns.

```
                T=000.50 A=000670.0( 15%) B=000732.0( 15%) C=000754.0( 15%) R=1.090
                T=000.50 A=000334.0( 20%) B=000456.0( 15%) C=000434.0( 15%) R=1.157
                T=000.50 A=000400.0( 15%) B=000480.0( 15%) C=000512.0( 15%) R=1.200

                T=000.50 A=000460.0( 15%) B=000558.0( 15%) C=000570.0( 15%) R=1.173
                T=000.50 A=211804.0(0.7%) B=248008.0(0.7%) C=250060.0(0.7%) R=1.174
```

Perhaps the excess etOH inhibited the rxn, or there may also be substrate level inhibition

★ Idea: put in *DTTP as trace in d GTP fields. What will this tell?

30 minutes

TdT assay

1. 1λ of DNA-pUC13(HIII cl)/μg(λ) — 1μg
   1λ TdT — 3units
   1λ α-32P dTTP (Evaporated and reeluted)
   4λ Pool- — std
   18λ 50mM dTTP — 36nM dTTP
   25λ

2. Same as above but 1λ of .1μg/λ pUC13(HIII — .1μg

3. as above, but 1λ of .01μg/λ pUC13HIII .01μg

4. control
   NO DNA
   NO TdT
   23λ total
   ~~spot on gel~~
   dilute 1λ 1:10 then spot

5≡5. Check to see that such massive amts of dNTP's are still causing substrate-level inhibition

   1λ of 1μg/λ Pe DNA
   1λ TdT
   1λ α-32P dTTP
   4λ Pool-
   1λ ~~serial dilution of 50mM dTTP~~ ← .5mM
   of a 1:4 dilution of 50mM dTTP

   ~~Pool~~
   17λ H2O
   25λ total

Pool Spot: 1λ of 1:10 dilution ← from control (23λ total)

PROG 6 USER 1

SR CH 12 1 TIMES
CR 1 TIMES
SCR =OFF
AQC =OFF
SF =ON
CALC= 1
PST = 10.00 MIN
CH 1 2.00 2 SIGMA %
.0 LSR
.0 BKG
.00 2 SIGMA B
0 LL
397 UL
CH 2 2.00 2 SIGMA %
.0 LSR
.0 BKG
.00 2 SIGMA B
655 LL
940 UL

POS	CH 1	2S%	CH 2	2S%	TIME
8	156.4	5.0	5622.1	.8	10.00
9	22.9	13.2	472.8	2.9	10.00
10	22.7	13.2	316.9	3.5	10.00
11	3624.6	1.9	202151.8	.2	2.76
12	18.4	14.7	235.9	4.1	10.00
13	281.3	3.7	9136.4	.6	10.00

Calculations for above experiment

1. 1 λ of 1 µg/λ DNA

$$\frac{GTP}{PUC13} = \frac{10\ mM}{4.44\times10^{-5}\ uM} = 2.25\times10^{5}$$ ← max possible incorporation

fraction of counts incorporated

$$\frac{5622.1-235.9}{(9136.4)(1.087\times10^{25})} = \frac{5386.2}{2,482,816.7} = .00217$$

(1.087 × 10^25 ← diluted 1:10; × 25 to get total possible counts)

$$\left(\frac{1}{23}\right)(25\lambda) = 1.087\lambda$$

↑ 1λ from a total volume of 23λ is equal to 1.087 from a final volume of 25λ

actual incorporation: $(.00217)(2.25\times10^{5}) = 488\ T/3'end$

2. 1 λ of .1 µg/λ DNA

$$\frac{GTP}{PUC13} = \frac{10\ mM}{4.44\times10^{-6}\ uM} = 2.25\times10^{6}$$ ← max possible incorp

fraction of counts incorporated

$$\frac{478.2-235.9-242.3}{2,482,816.7} = 9.76\times10^{-5}$$

actual incorp → $(9.76\times10^{-5})(2.25\times10^{6}) = 220\ T/3'end$

B. 1λ of .01μg/λ Puc[13]/H

max possible incorp → 2.25×10^7

fraction of counts incorp → $\frac{3169 - 235.9}{2,482,816.7} = 3.26 \times 10^{-5}$ (=81)

actual incorp → $(2.25 \times 10^7)(3.26 \times 10^{-5}) = 734$

S. = S 1λ of 1μg/λ Puc[13]/H [illegible] – diff conc of dTTP

• molarity of dTTP $(\frac{1}{100})(50mM) = .5mM$

max possible incorp = $\frac{dTTP}{3'OH} = \frac{.5mM}{4.44 \times 10^{-5}mM} = 1.13 \times 10^4$

fraction of counts incorp → $\frac{202,151.8 - 235.9}{2,482,816.7} = .0813$ (=201,915.9)

actual incorp → $(.0813)(1.13 \times 10^4) = 919$ T/3' end

TdT Assay

1. 4λ pool — st'd
   1λ pUC13/HIII @ 1μg/λ — 1μg
   5λ ^{3}H dGTP-evap & reeluted
   1λ TdT — ~3 units
   5λ 1:10 dilution of — 1mM
   50mM dGTP
   9λ H_2O
   25λ

2. 4λ pool
   1λ pUC13/HIII
   5λ ^{3}H dGTP-evaps reeluted
   1λ TdT
   10λ 1:10 dilution of 50mM dGTP — 2mM dGTP
   4λ H_2O
   25λ

3. Same as above, but
   2.5λ 1:10 dil. of 50mM dGTP — 0.5mM dGTP
   11.5λ H_2O
   25λ

4. 1λ 1:10 dil. of 50mM dGTP — 0.2mM dGTP
   13λ H_2O
   25λ

200λ of ^{3}H dGTP was vac dryed & reeluted in 40λ TE (⇒ 5X concentration)

~~4λ~~ H_2O

4λ Buc 13/11 III @ 1μg/λ 1μCi
1λ TdT ~3 units
1λ α-32P dTTP
4λ Pool^{-1}
2λ of a 1:4 dilution of 50mM dTTP ⇒ 1mM dTTP

run for: 25λ total
10Min
30Min ⇒ 4 separate runs
1hour
2hour

← NO DNA ~~added~~ TdT

+ Control (23λ total)
+ pool-spot 1λ from control
+ "Save" 30Min for the sequencing experiment

On "Save" - don't stop or acid wash - stop by chilling - it will later be phenol extracted & then cut & run out on gel

10Min 005 T=000.50 A=258694.0(0.7%) B=297376.0(0.7%) C=303772.0(0.7%) R=1.151

30Min 006 T=000.50 A=273180.0(0.7%) B=375920.0(0.5%) C=332902.0(0.5%) R=1.193
60Min 007 T=000.50 A=300768.0(0.7%) B=353172.0(0.5%) C=365600.0(0.5%) R=1.190
2hr 008 T=000.50 A=195122.0(0.7%) B=219508.0(0.7%) C=223372.0(0.7%) R=1.125
Control 009 T=000.50 A=000664.0(.15%) B=000768.0(.15%) C=000856.0(.10%) R=1.151
010 T=000.50 A=151398.0(1.0%) B=200524.0(0.7%) C=207264.0(0.7%) S=1.151

Could pool 1λ (+dil.) from orig. 23λ control

Remember, the calculated values are per 3' end

30 minutes

$\frac{dTTP}{3'OH} = \frac{1mM}{4.44\times10^{-5}mM} = 2.25\times10^4$

$\frac{273,180-664}{(151,398)(\frac{25}{23})(25)} = \frac{272,516}{4,114,076.1} = .0662$

$(.0662)(2.25\times10^4) = 1,490$ T/3' end

~~10~~ 20 minutes

$\left(\frac{258,694-664}{4,114,076.1}\right)(2.25\times10^4) = 1,411$ T/3' end

1 hour

$\left(\frac{300,768-664}{4,114,076.1}\right)(2.25\times10^4) = 1,641$ T/3' end

2 hours

$\left(\frac{195,122-664}{4,114,076.1}\right)(2.25\times10^4) = 1064$ T/3' end

check to see if EtOH in this

idea: in plastic bag put {Pool- 1:10 dil of all 4 bases

- pound/EtOH the sample for the gel
- try all 4 in labelling rxn

TdT Assay w/ all 4 bases present

1. 1λ	Puc 13/HIII DNA @ 1μg/λ	1μg
1λ	TdT @ ~3U/λ	3U
4λ	Pool-	St'd
1.3λ	1:10 dil of 50mM d6TP (=5mM)	0.25mM d6TP
1.3λ	1:10 dil of 50mM dCTP (=5mM)	0.25mM dCTP
1.3λ	1:10 dil of 50mM dATP (=5mM)	0.25mM dATP
1.3λ	1:10 dil of 50mM dTTP (=5mM)	0.25mM dTTP
1λ	α-32P dTTP (evap & reeluted)	32P-T-label
5λ	3H d6TP (evap & reeluted)	3H-G-label
7.8λ	H_2O	
25λ	total	

2. 1λ	Puc 13/HIII DNA @ 1μg/λ	1μg
1λ	TdT @ ~3U/λ	3U
4λ	Pool-	St'd
1.3λ	1:10 dil of 50mM d6TP	0.25mM d6TP
1.3λ	" dCTP	" dCTP
1.3λ	" dATP	" dATP
1.3λ	" dTTP	" dTTP
1λ	α-32P dCTP	32P-C-label
5λ	3H dATP (evap & reeluted)	3H-A-label
7.8λ	H_2O	
25λ	total	

C1 ≡ 3. Control - for rxn #1

4λ	Pool-
1.3λ	1:10 dil of 50mM d6TP
1.3λ	" dCTP
1.3λ	" dATP
1.3λ	" dTTP
1λ	α-32P dTTP
5λ	3H d6TP
9.9λ	H_2O

C2 ≡ 4. Control - for rxn #2

4λ	Pool-
1.3λ	d6TP
1.3λ	dCTP
1.3λ	dATP
1.3λ	dTTP
1λ	α-32P dCTP
5λ	3H d6TP
9.9λ	H_2O

From each of the controls spot 17 as the "pool"

1, 2, C1, C2, P1, P2

→ 40 min

INCORP of G

$$\frac{\text{Max possible G incorp}}{\text{3' end}} \rightarrow \frac{dGTP}{} = \frac{0.25\,mM}{4.44\times10^{-5}\,mM} = 5{,}631$$

$$\frac{\text{fraction of counts incorp}}{} \rightarrow \frac{136{,}890 - 570}{(122{,}262)(25)} = .0446$$

$$\frac{\text{actual incorp}}{} \rightarrow (.0446)(5{,}631) = 251$$

INCORP of A

$$\frac{\text{max possible incorp}}{} \rightarrow \frac{.25\,uM}{4.44\times10^{-5}\,\mu M} = 5{,}631$$

$$\frac{139{,}538 - 584}{(131{,}524)(25)}(5{,}631) = 238$$

incorp of C

$$\frac{201{,}442 - 926}{(412{,}726)(25)}(5{,}631) = 109$$

incorp of T

$$\frac{47{,}096 - 266}{(113{,}821)(25)}(5{,}631) = 93$$

	base/3kcal	%
A	238	34.4%
C	109	15.8%
G	251	36.3%
T	93	13.5%
	691	100%

$\Rightarrow G > A > C > T$

T=001.00 A=000570.0(10%) B=000266.0(15%) C=000999.0(7.0%) R=0.456
T=001.00 A=000534.0(10%) B=000926.0(7.0%) C=001332.0(5.0%) R=1.526
T=001.00 A=136990.0(0.7%) B=047096.0(1.0%) C=226411.0(0.5%) R=0.348
T=001.00 A=139533.0(0.7%) B=201462.0(0.5%) C=416207.0(0.3%) R=1.447
T=001.00 A=122262.0(0.7%) B=113821.0(0.7%) C=302295.0(0.5%) R=0.931
T=001.00 A=131524.0(0.7%) B=419726.0(0.3%) C=705104.0(0.3%) S=0.931

A: $1/2\ {}^3H$ B: β from ^{14}C only C: $^3H + {}^{14}C + \beta$s

Ran over again, exactly the same, EX the exception of #2, which will just be stopped & run out on a gel
↑ two #2's

~~2:55~~ 40 minutes, as before

[redacted]

```
#6  T=001.00  A=000959.0(3.0%)  B=000357.0( 1.5%)  C=001562.0(7.0%)  R=0.368
#5  T=001.00  A=000549.0( 10%)  B=000516.0( 10%)  C=001333.0(7.0%)  R=0.944

#6  T=001.00  A=281265.0(0.5%)  B=069662.0(1.0%)  C=432974.0(0.3%)  R=0.247
#A  T=001.00  A=174665.0(0.5%)  B=279508.0(0.5%)  C=551349.0(0.3%)  R=1.600
#C  T=001.00  A=177395.0(0.5%)  B=113914.0(0.7%)  C=346391.0(0.5%)  R=0.642
#T  T=001.00  A=183277.0(0.5%)  B=324967.0(0.5%)  C=593820.0(0.3%)  S=1.773
```

same channels as above

⇓

[6] → $\frac{281,265-959}{(177,395)(25)}(5,631) = 356$

[A] → $\frac{174,665-549}{(193,277)(25)}(5,631) = 214$

[C] → $\frac{279,508-516}{(324,967)(25)}(5,631) = 193$

[T] → $\frac{69,662-357}{(113914)(25)}(5,631) = 137$

		%
G	356	40%
A	214	24%
C	193	21%
T	137	15%
Total	900	

↳ G > A > C > T

The saved portion from the pcr of T's tailing (0.15 pellet) was frozen after the rxn was completed (30 min). Then, together w/ the "saved portion" from the experiment immediately above, the two were phenol extracted & EtOH ppt'd

↓ re-eluted

each, ~~12.5λ~~ ~~60λ~~ H_2O ← ~1 µg in rxn mix
1λ SphI = 5 units
1.5λ 10x Low salt buffer
15λ total

37°C @ 8:40 am

↳ 2 hours, 20 mins long

↳ both of these samples are loaded on a sequencing gel run by Clark

-Calculations of Stop codon appearance based on base composition

Term. codons: UAA UGA ⇒ TAA TGA
UAG TAG

Using Data from the first experiment w/ all 4 n.t.'s present

$(T)(A)(A) + (T)(A)(G) + (T)(G)(A)$ where T = prob of T = .135
A = etc. = .344
C = .158
G = .363

~~$(.135)(.344)^2 + (.135)(.344)(.363) + (.135)$~~

$= (T)(A^2) + 2(T)(A)(G)$

$= (.135)(.344)^2 + (2)(.135)(.363)(.344)$
$= .04969$

# of residues	Fraction terminated		
1	.95	.983	.983 .98
10	.60	.843	.843 .82
30	.22	.60	.60 .56
50	.078	.422	.422 .38
75	.022	.279	.279 .22
100	.006	.182	.182 .14
150	.00047	.078	.078 .05
200	.000037	.033	.033 .02
300	.0000002	.006	.006 .03

A = .15
T = .15
C = .35
G = .35

$(.15)(.15)^2 + 2(.15)(.15)(.35)$

A = .15
T = .15
C = .40
G = .30

Anticipated N.T. concentrations

A: ~~(.34/.15)(.25mM)~~ =

desired → (.15/.34)(.25mM) = .11mM ← actual

T: (.15/.135)(.25mM) = .28mM

C: (.40/.158)(.25mM) = .63mM

G: (.30/.363)(.25mM) = ~~.21mM~~ .21mM

1.23mM total [N.T.]

these values are calculated per mg of DNA

TdT Assay (Try out previously calculated concentrations & check the new Pharmacia terminal transferase

1. 1λ [redacted] PUC13/HIII DNA @ 1μg/λ
   1λ lab TdT (~30units/λ)
   4λ pool
   2.2λ 1:40 dil of 50mM dATP (=1.25mM)
   1.4λ 1:10 dil of 50mM dTTP (=5mM)
   3.2λ 1:10 dil of 50mM dCTP (=5mM)
   1.1λ 1:10 dil of 50mM dGTP (=5mM)
   1λ α-32P dTTP
   3λ 3H dGTP
   7.1λ H_2O

   [redacted] 1μg
   30units
   St'd Concentration
   .11mM dATP
   .28mM dTTP
   .63mM dCTP
   .21mM dGTP

2. [redacted] same as above except
   1λ α-32P dCTP
   3λ 3H dATP
   25λ

3. Control for rxn 1
   as above #1 but no DNA or TdT
   ↳ 9.1λ H_2O
   25λ

4. Control for rxn 2
   as #2 but no DNA or TdT
   ↳ 9.1λ H_2O
   25λ

~~P-L~~ TdT
repeat above: [redacted] use same control;
1 & 2 use just 0.5λ TdT
and compensate w/ 7.6λ H_2O
↳ 1' & 2' than labelled

Take 2λ from each control and spot to use as a "std" spot

~~1:45~~ → 40 minutes

Stock ^{3}HdATP was diluted, so that there'd be enough for this experiment

[redacted]

Same channels as before

A= [redacted]
B= [redacted]
C= [redacted]

Lab Talk

[A] $\frac{dATP}{3'} = \frac{.111mM}{4.44\times10^{-5}mM} = 2,478$

$\frac{13,948-166}{29,454\ (12.5)}(2,478) = 93$

[G] $\frac{dGTP}{3'} = \frac{.21mM}{4.44\times10^{-5}mM} \cong 4,730$

$\frac{221,554-292}{239,414\ (12.5)}(4,730) = 363$

[C] $\frac{dCTP}{3'} = \frac{.63mM}{4.44\times10^{-5}mM} \cong 14,189$

$\frac{494,348-888}{199,174\ (12.5)}(14,189) = 801$

T $\frac{dTTP}{3'} = \frac{.28mM}{4.44 \times 10^{-5} mM} = 6,306$

$\frac{60,312 - 284}{278,562 (12.5)} (6,306) = 109$

P-L TdT

A $\frac{2,472 - 166}{29,454 (12.5)} (2,478) = 16$

G $\frac{31,372 - 292}{230,414 (12.5)} (4,730) = 51$

C $\frac{22,920 - 888}{699,174 (12.5)} (14,189) = 117$

T $\frac{8,898 - 284}{278,562 (12.5)} (6,306) = 16$

	P-L #	P-L %	Lab Prog #	Lab Prog %	Calculat Goal
A	16	8%	93	7%	15
C	117	59%	801	59%	40
T	16	8%	109	8%	15
G	51	26%	363	27%	30
total	200		1366		

appearance of term. codons & above base ratios

$(T)(A^9) + 2(T)(A)(6)$

$(.08)(.08)^2 + (2)(.08)(.08)(.26)$

residues	% unstopped	
1	.9962	
50	.825	
100	.68	
150	.56	
200	.46	
250	.38	
300	.32	
350	.26	
400	.21	
500	.15	← 455 ⇒ 17% still untermicated (readthrough overlight synthesized)
600	.10	
700	.07	
1000	.02	

⇒ look to see how aa distribution ratio is affected

A New calculating anticipated [dNTP] to get the desired A=.15 (chosen on p. 19, white)
T=.15
C=.40
G=.30

A: $\frac{.15}{.08}(.11) = .24\,mM$ ← though I know that this conc. ratio before gave me too much

T: $\frac{.15}{.06}(.28) = .53\,mM$

C: $\frac{.40}{.59}(.63) = 1.42\,mM$

G: $\frac{.30}{.27}(.21) = \frac{.23\,mM}{1.42\,mM}$

I think that the problem w/ this sort of calculation is that one is dependent upon the other so that these should be "normalized" w/respect to each other in any calculation

target

desired goal:
A = .05
C = .25
G = .25
T = .45

This is one way to calculate expected concentrations

& also one way to run out on a gel

last experiment →

	fraction = product		main incorp fraction	rxn fraction / product fraction = M
A = .07	.11 mM		.082	.082/.07 = 1.17
C = 0.59	.63 mM		.47	.47/.59 = .80
T = .08	.28 mM		.209	.209/.08 = 2.61
G = .27	.12 mM		.157	.157/.27 = .581
	1.34 mM			

desired product fraction	rxn fraction (estim.) = M (desired product fraction)
A = .05	.0684
C = .25	.20
G = .25	.653
T = .45	.261
	1.18

⇓

normalize w/ respect to tot

A	.0684/1.18 = .058
C	.20/1.18 = .17
G	.653/1.18 = .55
T	.261/1.18 = .22
	.998

1. 1λ Pst 13 1 HIII DNA @ 1μg/λ
   1λ TdT
   4λ Pool
   1.2λ 1:40 dil of 50mM dATP (=1.25mM)
   3.4λ 1:40 dil of 50mM dCTP (=1.25mM)
   2.8λ 1:10 dil of 50mM dGTP (=5mM)
   1.1λ 1:10 dil of 50mM dTTP (=5mM)
   1λ α-32P dTTP
   3λ 3H dGTP
   6.5λ H_2O
   25λ

1μg
~3 units,
Std concentrations
~~.058mM~~ dNTP ~~.086~~
.17mM dCTP
~~.15~~ mM dGTP
.22mM dTTP

2. Same as above but
   1λ α-32P dCTP
   3λ 3H dATP

Save ≡ 1

For controls pool before adding TdT to the 3 rxn mixtures draw out 2λ and spot to count - replace w/ 2λ of H_2O. So, there'll be a C1
C1' (= Save)
C2

Another way to calculate out expected concentrations

Via ratio of dNTP/3'

		[dNTP]/[3']		anticipated [dNTP]
A=.07	.11mM	2,478	.05/.07(2,478) = 1,770	.079mM
C=.59	.63mM	14,189	.25/.59(14,189) = 6,012	.27mM
T=.08	.28mM	6,306	.25/.08(6,306) = 19,706	.87mM
G=.27	.21mM	4,730	.45/.27(4,730) = 7,883	.35mM

↑ total tail length
1366

total tail length of 1,000 would be better, so multiply all [dNTP]'s by 1,000/1366 (=.732)

	[dNTP]
A	.058mM
C	.20mM
T	.64mM
G	.26mM
	1.16mM

1, 8 1λ PvuII 3'H-III DNA @ 1 ng/λ
1λ TdT
4λ pool
1.2λ 1:40 dil of 50mM dATP
1λ 1:10 dil of 50mM dCTP
3.2λ 1:10 dil of 50mM dTTP
1.3 1:10 dil of 50mM dGTP
1λ α-32P dTTP
3λ 3H dGTP
+ 2λ H_2O
25λ total

1 ng
~3 units

		40
	50mM dCTP	20mM dC
	50mM dTTP	10mM dT
	50mM dGTP	6mM dG

2 Same as above, but
1λ α-32 dCTP
3λ 3H dATP

3 Same = 2' (same as 2)

For pool spot: Just prior to adding the TdT withdraw 2λ of rxn and spot. Then to restore original concentration add 2λ of H_2O. Then add TdT. (make sure everything well mixed)

3 pool spots
P1
P2'(= Sam2)
P2

40 minutes

The above isn't such a good way to do this because there is no background level produced from the neg. cont
(I also forgot to save "save" & acid ppt it by mistak

[redacted]

001 T=000.50 A=228682.0(0.7%) B=099116.0(1.0%) C=398834.0(0.5%) R=0.993
002 T=000.50 A=010042.0(3.0%) B=650738.0(0.5%) C=792653.0(0.5%) R=5.040
003 T=000.50 A=005706.0(5.0%) B=442938.0(0.5%) C=506555.0(0.5%) R=9.071
004 T=000.50 A=230364.0(0.7%) B=287778.0(0.7%) C=675748.0(0.5%) R=1.249
005 T=000.50 A=015394.0(3.0%) B=842870.0(0.3%) C=141420.0(0.3%) R=5.447
006 T=000.50 A=009122.0(3.0%) B=681974.0(0.5%) C=854122.0(0.3%) R=

[redacted]

[A]

$\frac{dNTP}{3'} = \frac{.06mM}{4.44 \times 10^{-5} uM} = 1351$

$2 \Rightarrow \frac{10,212}{(5,341)(12.5)} (1351) = 71$

$2' \Rightarrow \frac{5,706}{(122)(12.5)} (1351) = 69$

[C] $\frac{dNTP}{3'} = \frac{.20}{4.44 \times 10^{-5}} = 4,505$

$2' \Rightarrow \frac{650738}{442,870(12.5)} (4,505) = 278$

$2' \Rightarrow \frac{442,938}{681,974(12.5)} (4,505) = 224$

[G] $\frac{dGTP}{3'} = \frac{.26}{4.44 \times 10^{-5}} = 5,85$

$\frac{228,682}{230,364(12.5)} (5,856)$

≈ 465

[T] $\frac{dTTP}{3'} = \frac{.164}{4.44 \times 10^{-5}} = 14,41$

$\frac{99,116}{287,778(12.5)} (14,414)$

$= 397$

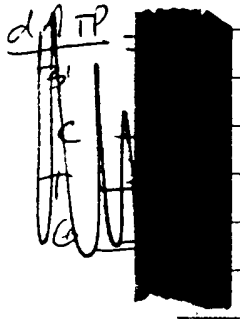
	#	%	Corrected
A	70	5.90%	5
C	251	21.26	25
G	468	39.59%	25
T	397	33.59%	45
	1186	160.19%	

[redacted]

A = .059 C = .211 G = .395 T = .335

[redacted]

```
term =  1.678049E-02
ala =   .083345
arg =   9.392546E-02
asn =   1.900626E-03
asp =   1.272453E-02
cys =   7.224946E-02
gln =   5.651846E-03
glu =   1.058047E-02
gly =   .156025
his =   6.797154E-03
ile =   1.195783E-02
leu =   .1216352
lys =   1.580374E-03
met =   7.807176E-03
phe =   6.127485E-02
pro =   .044521
ser =   8.340953E-02
thr =   .012449
trp =   5.226838E-02
tyr =   1.079169E-02
val =   .132325
```



[redacted]

TdT Tailing

Goal: In the large scale tailing it would be more convenient to use more concentrated DNA & dNTP, while maintaining the same dNTP/3'OH ratio. This has the advantage of a smaller total rxn volume and the requirement for less TdT. Therefore, this TdT assay will use greater concentrations.

Also, it would be nice to compare tailed vs. untailed plasmid on an agarose gel. Some of the DNA from this rxn will be run on a gel

Procedure

1. 2 λ PUC13/III^{III} @ 1 μg/1 λ	2 μg DNA
4 λ pool	Std
1 λ of 1:5 dil of TdT	(This makes for 1/10 the amt used in prior assay)
2.3 λ 1:40 dil of 50mM dATP	.115 mM dATP
2 λ 1:10 dil of 50mM dCTP	.4 mM dCTP
6.4 λ 1:10 dil of 50mM dTTP	1.28 mM dTTP
2.6 λ 1:10 dil of 50mM dGTP	.52 mM dGTP
1 λ α-^{32}P dTTP	
3.7 λ ^{3}H dGTP	
25 λ	

2. Same as above but
1 λ α-^{32}P dCTP
3.7 λ ^{3}H dATP
25 λ total

1:40 pm

Before adding the TdT, withdraw 2λ and spot on disk to count. (Do not replace with 2λ H_2O – if the mixture is homogenous before withdrawl then the concentrations will remain the same; the total volume will just decline slightly.)

After the rxn goes for its designated length withdraw 8λ from each rxn mixture ($\frac{8}{25}$(2μg) = .64μg DNA) to run out on gel. Then stop rxn and count ppt as before.

Remember, in the calculations, only $\frac{23-8}{25}$(2μg) = 1.2μg will remain

1 026 T=000.50 A=012040.0(0.0%) B=011006.0(3.0%) C=023354.0(2.0%) R=0.95
2 027 T=000.50 A=013036.0(0.0%) B=010900.0(1.5%) C=024220.0(1.5%) R=2.48
P1 028 T=000.50 A=020076.0(1.5%) B=030040.0(0.5%) C=051008.0(0.3%) P=3.705
P2 029 T=000.50 A=020076.0(0.7%) B=030046.0(0.2%) C=050942.0(0.3%) S=1.705

Remainders only 237-87 = 150 was counted

A $\frac{dNTP}{3'OH} = \frac{.115\,uM}{8.85\times10^{-5}\,uM} = 1295$

$\frac{18086}{293,886}(1295) = 11$ (7.5)

C $\frac{dNTP}{3'OH} = \frac{.4\,uM}{8.85\times10^{-5}\,uM} = 4,505$

$\frac{45002}{479,946}(4505) = 31$ (7.5)

T $\frac{dNTP}{3'OH} = \frac{1.28\,uM}{8.85\times10^{-5}\,uM} = 14,414$

$\frac{11096}{526,960}(14,414) = 40$ (7.5)

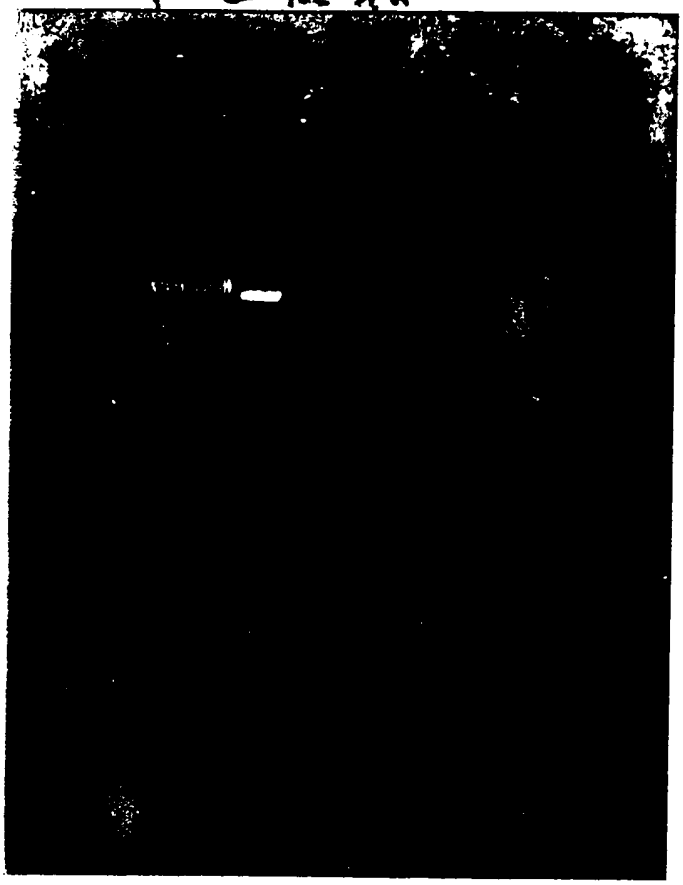
G $\frac{dNTP}{3'OH} = \frac{.52\,uM}{8.85\times10^{-5}\,uM} = 5,856$

$\frac{12940}{135,996}(5,856) = 73$ (7.5)

	#	%
A	11	7%
C	31	20%
G	73	47%
T	40	26%
	155	100%

Remainders

1 2 Pulse 1 3/4 III



A = .07 C = .2 G = .47 T = .26

term = .018382
ala = 9.399999E-02
arg = .111766
asn = .002254
asp = .015134
cys = .056212
gln = 7.560001E-03
glu = .017766
gly = .2209
his = .00644
ile = 9.645999E-03
leu = .088504
lys = .002646
met = 8.553999E-03
phe = .031096
pro = .04
ser = .067134
thr = .014
trp = .057434
tyr = .008372
val = .1222

Goal: - find out min. volume of enzyme
- get DNA for gels
- get DNA for HPLC

1λ 1λ PUC13/HIII @ 1μg/λ — 1μg std
4λ Pool
1.2λ 1:40 dil of 50mM dATP — .06 mM dATP
1λ 1:10 dil of 50mM dCTP — .20 mM dCTP
3.2λ 1:10 dil of 50mM dTTP — .64 mM dTTP
1.3λ 1:10 dil of 50mM dGTP — .26 mM dGTP
1λ α-32P dTTP
3λ 3H dGTP
-TdT
-H_2O
25λ total

~~10λ TdT undil~~
~~3λ H_2O~~

rxn went for 1½ hours

2 same as 1 but,
1λ α-32P dCTP
3λ 3H dATP

(A)	(B)	(C)	(D)
1λ TdT, undil	5λ 1:10 dil TdT	3λ 1:10 dil TdT	2λ 1:10 dil
8.3λ H_2O	4.3λ H_2O	6.3λ H_2O	7.3λ H_2O

Withdrew 2λ from the rxn mix before adding TdT and spot.

~~After the rxn ends withdrew 13λ saved for~~ HPLC

labelled: A-1 B-1 C-1 D-1
A-2 B-2 C-2 D-2

Aliquots of 12λ were withdrawn from each A-1 & A-2 (6λ on gel, 6λ saved for HPLC) Nothing was withdrawn from B, C, or D

A-1 A-2 CH HSE

I gaged: B-2 & A-2 were washed through the filter before they were adapted ⇒ very few units were absorbed

1: A = H-dGTP, B = [illegible]

2: A = ^{3}H-dATP, B = ^{32}P-dCTP

[illegible]	T=000.50	A=[illegible]	(2.0%)	B=[illegible]	(1.0%) C=153972.4(1.0%) R=3.600
[illegible]	T=000.50	A=[illegible]	(3.0%)	B=[illegible]	(2.0%) C=056230.0(1.5%) R=2.571
[illegible]	T=000.50	A=[illegible]	(2.0%)	B=[illegible]	(1.5%) C=113114.3(1.0%) R=2.714
[illegible]	T=000.50	A=[illegible]	(1.5%)	B=[illegible]	(0.7%) C=317160.0(0.7%) R=2.594
[illegible]	T=000.50	A=[illegible]	(3.0%)	B=[illegible]	(1.5%) C=020590.0(1.5%) R=3.714
[illegible]	T=000.50	A=[illegible]	(1.0%)	B=[illegible]	(1.0%) C=242673.6(0.7%) R=0.594
[illegible]	T=000.50	A=[illegible]	(3.0%)	B=[illegible]	(2.0%) C=047170.8(1.5%) R=1.714
[illegible]	T=000.50	A=[illegible]	(1.5%)	B=[illegible]	(1.0%) C=271755.6(0.7%) R=3.000
[illegible]	T=000.50	A=[illegible]	(0.7%)	B=[illegible]	(0.7%) C=[illegible]
[illegible]	T=000.50	A=[illegible]	(1.5%)	B=[illegible]	C=[illegible]
[illegible]	T=000.50	A=[illegible]	(0.7%)	B=[illegible]	C=[illegible]
[illegible]	T=000.50	A=[illegible]	(1.5%)	B=[illegible]	C=[illegible]
[illegible]	T=000.50	A=[illegible]	(0.7%)	B=[illegible]	C=[illegible]
[illegible]	T=000.50	A=[illegible]	(0.7%)	B=[illegible]	C=[illegible]
[illegible]	T=000.50	A=[illegible]	(1.5%)	B=[illegible]	C=[illegible]
[illegible]	T=000.50	A=[illegible]	(0.7%)	B=[illegible]	C=[illegible]
[illegible]	T=000.50	A=[illegible]	(0.7%)	B=[illegible]	C=[illegible]

Rxn A

(A) $\frac{(16902)(2)}{(192,833)(13)}(1351) = 18$ ✓ unreliable due to gaps

(C) $\frac{(38,495)(2)}{(646,835)(13)}(4505) = 42$ ✓ unreliable due to gaps

(G) $\frac{(27340)(2)}{(41,680)(13}$ ← $(5856) = 591$

(T) $\frac{(106581)(2)}{(329,711)(13)}(14,414) = 717$

Rxn B:

(A) $\frac{66,584(2)}{244,030(23)}(1351) = 3$ ✓ unreliable-poor

(C) $\frac{(192,833)(2)}{(813,210)(23)}(4,505) = 93$ ✓ unreliable-poor

(E) $\frac{(25,160)(2)}{(49,750)(23)}(5856) = 258$

(F) $\frac{(69,675)(2)}{(406,142)(23)}(14,414) = 215$

Rxn C:

(A) $\frac{(15,156)(2)}{274,033)(23)}(4,505) = 79$

(E) $\frac{107,580)(2)}{14,000)(23)}(1351) = 59$

(G) $\frac{8,734)(2)}{2,560)(23)}(5856) = 224$

(I) $\frac{10,464)(2)}{26280)(23)}(14414) = 190$

Rxn D

(A) $\frac{72,084\ (2)}{316,971\ (23)}(1351) = 27$

(C) $\frac{(58686)(2)}{(71,080)(23)}(4505) = 87$

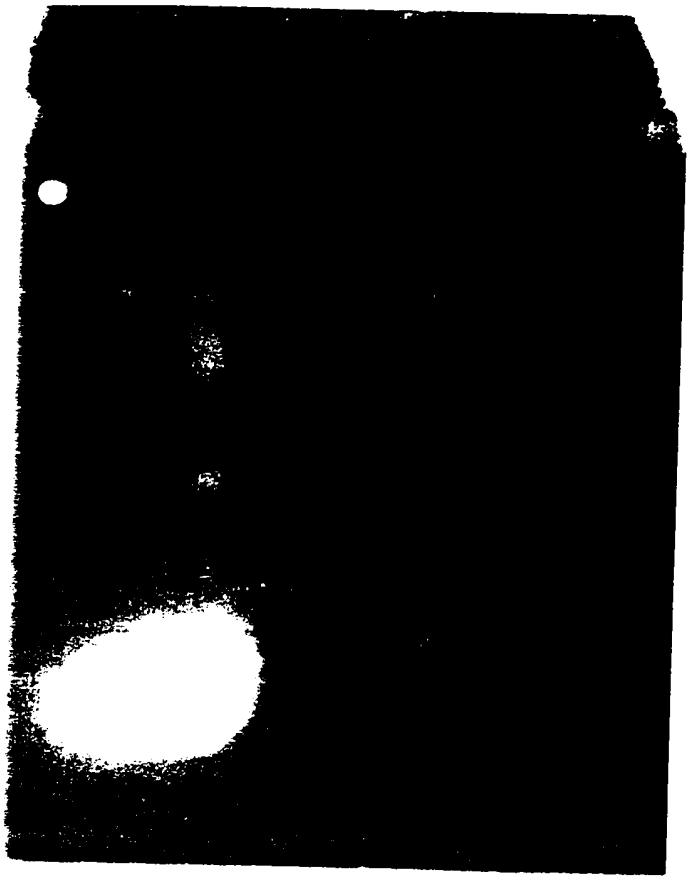
(G) $\frac{(14,430)(2)}{(41,504)(23)}(5856) = 177$

(T) $\frac{(25,521)(2)}{(97,240)(23)}(14,414) = 162$

Results

dNTP	Rxn A #	Rxn A %	Rxn B #	Rxn B %	Rxn C #	Rxn C %	Rxn D #	Rxn D %
A	18*		3*		59	10.7	27	6.0
C	42*		93*		79	14.3	87	19.2
G	591		258		224	40.6	177	39.0
T	717 (sum: 1308)		215 (471 sum)		190	34.4	162	35.8
					552	100	453	100

* unreliable due to goofup



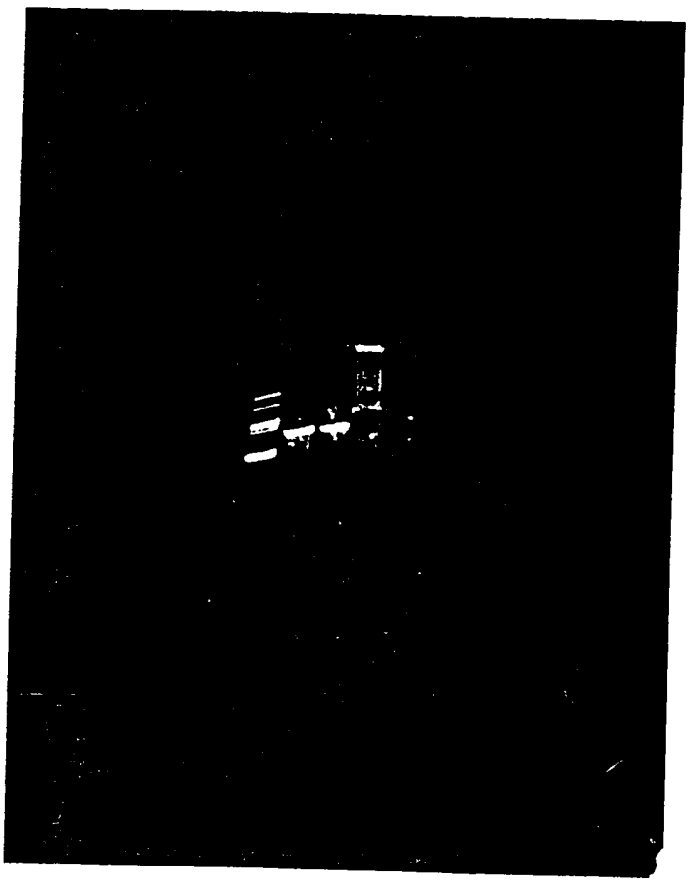
6 & hour autoradiograms of the above gel

PUC13 / HIII Digest

190λ PUC13 at 1μg/1λ (= 190μg)
33 ~~48~~λ HindIII at 4 u/λ (= ~~192~~ U)
26.4λ 10X "Core" buffer 1#Z
15λ H_2O
264.4λ total

Rxn went O/N @ 37°C

1given 200μg from Ray - 10μg/in 10λ) saved



After 12 hours the digest was stopped. Together w/ A-Z (from the days before) the DNA was ~~ethanol~~ EtOH precipitated.

The ~~DNA~~ PvuII/HincII was reeluted in 190λ TE

The A-Z was re-eluted & cut w/ ~~EcoRI~~ EcoRI cut 20u/λ

1λ EcoRI at 20u/λ (=20u)
~~EtOH~~ ppt & dry A-Z DNA
2λ 10x High Salt buffer
17λ H_2O
20λ total

Checking DNA concentration

	13/11 III - batch 1	13/11 III - batch 2
280	.29	.138
260	.378	.277

diluted 1:40 (5λ DNA + 195λ TE)

$\frac{40X}{22} = \frac{mg}{ml}$ ← 260 absorbance

($\frac{260}{280}$ ratio should be about 2, which it is)

- batch-1 .7 μg/λ
- batch-2 .5 μg/λ

} ⇒ tail length ~1.5x as long as longest

Large scale preparation for TdT step #1

Goal: To make all the reagents up in a sufficient quantity that identical preparations may be used for trial & actual step 1

Large scale set up of assay on p. 24 (white):

[redacted]

1 ml of Pool + dNTP at .06mM dATP
.20mM dCTP
.64mM dTTP
.26mM dGTP
} assuming stock concentrations at 50mM (not true) actual val

Stock		Final concentration in total volume of 1ml
100λ	1M K^+ Cacodylate	100mM Cacodylate
20λ	50mM $CoCl_2$	1mM $CoCl_2$
40λ	0.5M dith / 1:100 dil	0.2mM dith
12λ	50mM dATP / 1:10 dil	.06mM dATP
4λ	50mM dCTP	.20mM dCTP
12.8λ	50mM dTTP	.64 mM dTTP
5.2λ	50mM dGTP	.26mM dGTP
[redacted]		
6λ	H_2O	
200λ	total	

(concentrations are correct when tris pool is in a total volume of 1ml ⇒ 5X pool)

must add first
- label
- TdT
- DNA

To make 1ml total of the above pool - use 5 times the quantity

✓	500λ	KT Cacodylate
✓	100λ	$CoCl_2$
✓	200λ	1:100 dilution of dNTP
✓	60λ	1:10 dATP
✓	20λ	dCTP
✓	64λ	dTTP
✓	26λ	dGTP
✓	30λ	H_2O
	1000λ	(And this will be a 5x pool (+))

dNTP's individual

Add $CoCl_2$ last (at least after dilut)

TdT Assay

1.

- ✓ 5λ Pool++(5X)
- ✓ 1.4λ PuCl3(H^{III}-batch 2 at 0.5 μg/λ
- ✓ 0.5λ TdT
- ✓ 1λ $^{\alpha-32}$P dTTP
- ✓ 2λ ^{3}H dGTP
- ✓ 15.1λ H_2O

25λ total

2. same as #1, but

- ✓ 1λ $^{\alpha-32}$P dCTP
- ✓ 2λ ^{3}H dATP

Take 2λ out to spot before adding TdT

⇒ 2λ (taken out of 24.5)

12:05 pm

8λ withdrawn from both 1 & 2 before being stopped

⇒ 15λ total counted

PUC 19 MW = (649)(2686 bp) = 1,743,214 g/mole

~~[redacted]~~

1×10^{-6} g $\left(\frac{1 \text{ mole}}{1{,}743{,}214 \text{ g}}\right)$

1 µg = 5.74×10^{-13} moles

(2 3'OH per molecule)

⇒ 1.15×10^{-12} 3'OH M

in 1 µg

Saint Data on next page

A $\frac{dATP}{3'OH} = \frac{.06\text{nM}}{(.7)(4.44\times10^{-5}\text{nM})} = 1,931$

~~$\frac{10,380(2)}{(24,300)(15)} (1931) = 110$~~ $\frac{63,290(2)}{167,258(15)} (1931) =$

C $\frac{dCTP}{3'OH} = \frac{.20\text{nM}}{(.7)(4.44\times10^{-5}\text{nM})} = 6,435$

$\frac{241,348(2)}{511,642(15)} (6,435) = 405$

G $\frac{dGTP}{3'OH} = \frac{.26\text{nM}}{(.7)(4.44\times10^{-5})} = 8,366$

$\frac{(10,380)(2)}{(24,300)(15)} (8,366) = 476$

T $\frac{dTTP}{3'OH} = \frac{.64\text{nM}}{(.7)(4.44\times10^{-5}\text{nM})} = 20,592$

$\frac{39,378(2)}{193,762(15)} (20592) = 558$

	#	%	(actual) per minute
A	97	6.3%	5.9%
C	405	26.4%	21.1%

T=000.50 A=010320.0(3.0%) B=039373.0(2.0%) C=05[illegible]

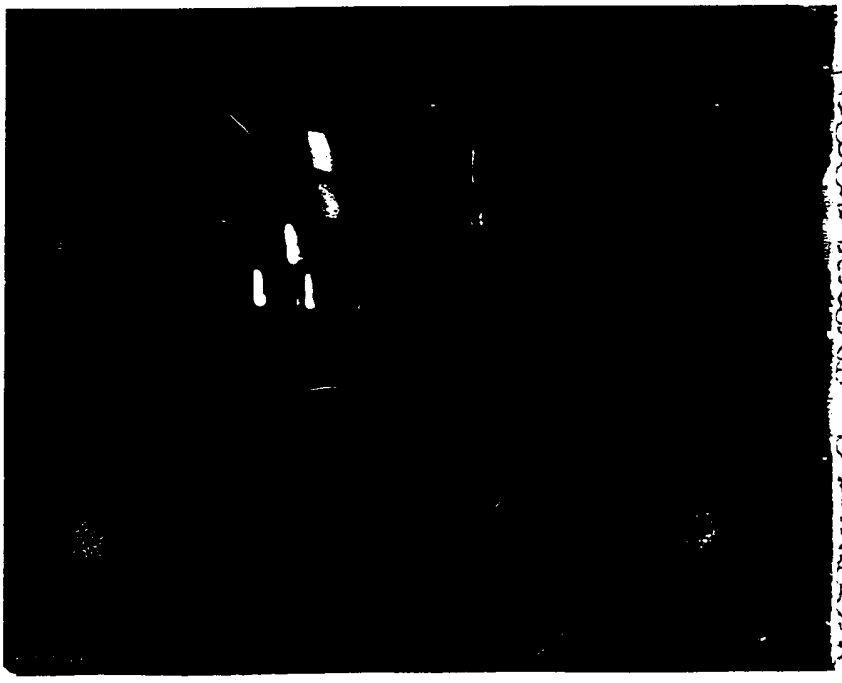
T=000.50 A=063290.0(1.5%) B=241343.0(0.7%) C=16[illegible]

T=000.50 A=024300.0(2.0%) B=193752.0(0.7%) C=[illegible]

T=000.50 A=167258.0(0.7%) B=511642.0(0.5%) C=[illegible]

Lamp

$\left.\begin{matrix} \\ \\ \end{matrix}\right\}$ - counts/pixel
- at most 200 counts/pixel

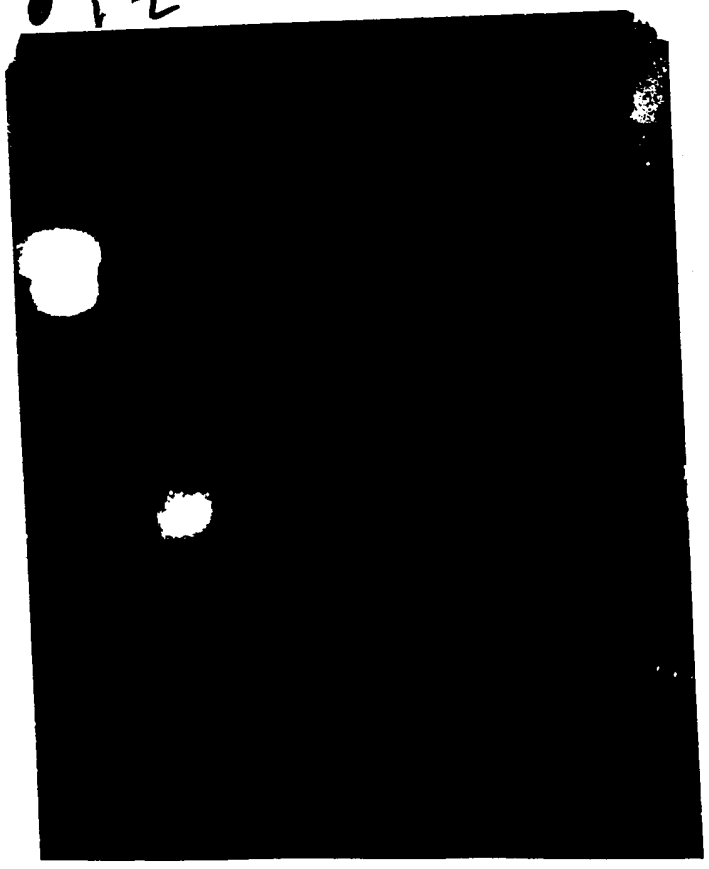


1 of 2
13/H III
13

A = .063 C = .264 G = .31 T = .363

term = 1.561953E-02
ala = .08184
arg = 8.912469E-02
asn = 2.488563E-03
asp = 1.224531E-02
cys = 7.055631E-02
gln = 6.203736E-03
glu = 7.28469E-03
gly = .0961
his = 1.042826E-02
ile = 1.577961E-02
leu = .1449818
lys = 1.480437E-03
met = 7.08939E-03
phe = 8.261916E-02
pro = 6.969601E-02
ser = .1080773
thr = .016632
trp = .0348843
tyr = 1.433886E-02
val = .11253

012



razor index

44 hour autoradiograms

TdT assay w/ less enzyme / pilot for large-scale prep

✓ 1.	5λ pool++ (5X)	st'd
✓	1.4λ PUC 13/H^{III} - batch 2 at 0.5μg/λ	.7μg
	1λ α-32 PdTTP	
	2λ ^{3}H dGTP	
	1λ 1:3 dil of TdT	
✓	14.6λ H_2O	
	25λ total	

2. Same as above, but
   1λ α-32 PdCTP
   2λ ^{3}H dATP

- Take 2λ out to spot before adding TdT
  ⇒ (2λ taken out of total volume of 24λ)

- Withdrew 8λ from each (×2 before stopping rxn ⇒ 15λ counted

rxn went for ½ hour

001 T=000.50 A=062396.0(1.5%) B=225948.0(2.0%) C=108428.0(
002 T=000.50 A=026084.0(2.0%) B=052510.0(1.6%) C=090744.0(
003 T=000.50 A=239512.0(0.7%) B=268618.0(0.7%) C=684198.0(
004 T=000.50 A=175398.0(0.7%) B=373326.0(0.5%) C=684198.0(

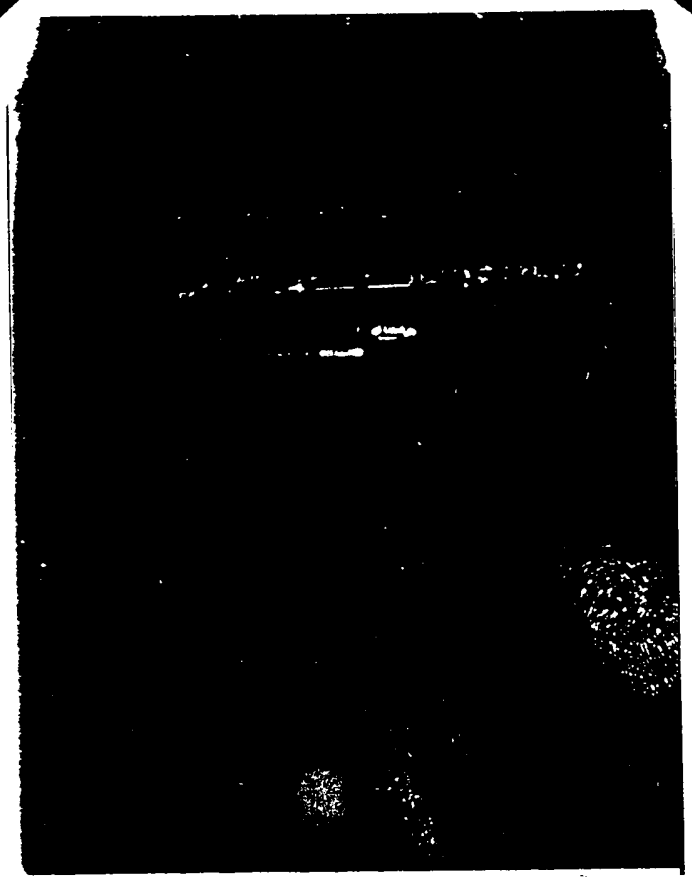
[A] $\frac{26,084\ (2)}{176,398\ (15)}\ (1931) = 38$

[C] $\frac{52,510\ (2)}{373,326\ (15)}\ (6,435) = 121$

[T] $\frac{62,396\ (2)}{239,512\ (15)}\ (8,336) = 290$

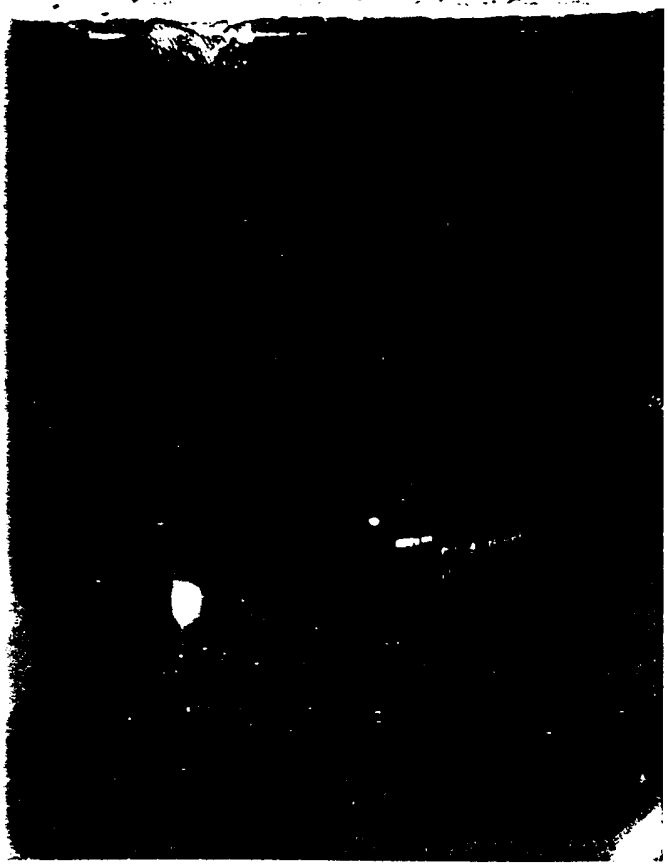
[T] $\frac{26,948\ (2)}{268,618\ (15)}\ (20,592) = 275$

	#	%
A	38	5.2%
C	121	16.7%
T	290	40.0%
T	275	38.0%



~~The sucrose IG should be ~3X as concentrated, but it does not look it. As~~

~3X as much cut added as sucrose



Large Scale Random TdT Tailing

175/1.4 = 125 x scale-u

3125 +

1562.5

ea

175λ pUC13/HIII at 0.5 μg/λ 87.5 μg DNA std

625 λ 5X pool++

50 λ TdT (1/2.5 of the 1λ in 25λ)

[illegible]

[illegible] pUC13/HIII at 0.5 μg/λ [illegible]

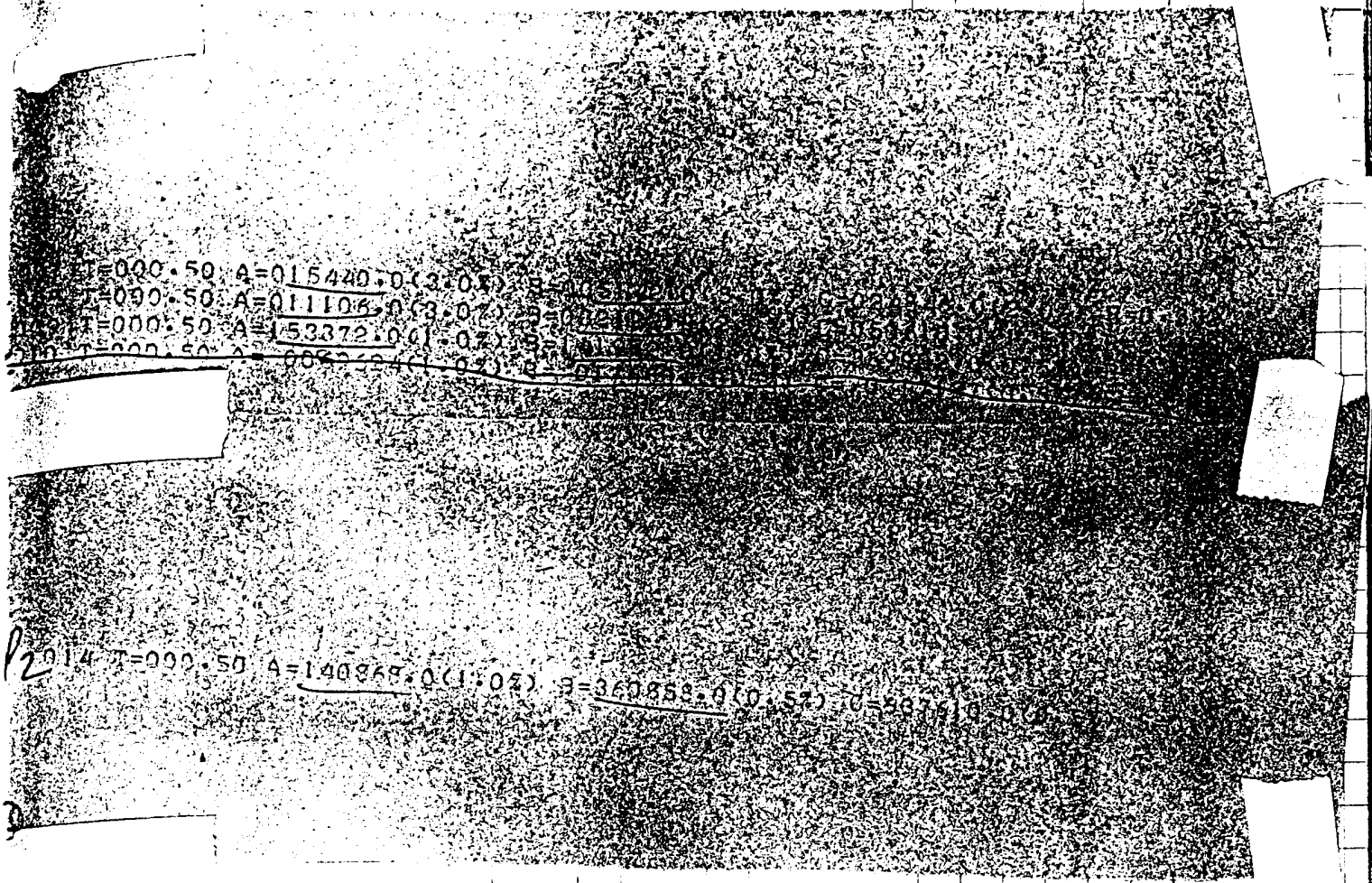
1. 87.5 λ pUC13/HIII at 0.5 μg/λ 43.75 std
2. 312.5 λ 5X pool++
3. 25 λ TdT (1/2.5 of the 1λ in 25λ)
4. 10 λ α-32 PdTTP
5. 20 λ 3H dGTP
6. 107.5 λ H_2O

1562.5 λ total

2. Same as above, but
10 λ α-32 PdCTP
20 λ 3H dATP

- Before Adding TdT withdraw [illegible] 10λ from each tube and spot
- After rxn ends (1 hour) withdraw 20λ from each tube - place [illegible] in culture tube - for counting
- withdraw 7λ and place in eppendorf - this is for gel
- The remaining bulk should be chilled / phenol extracted / [illegible] ppt.

Did I use the true concentration
of the dNTPs in this calculation
or did I just take them to be 50mM



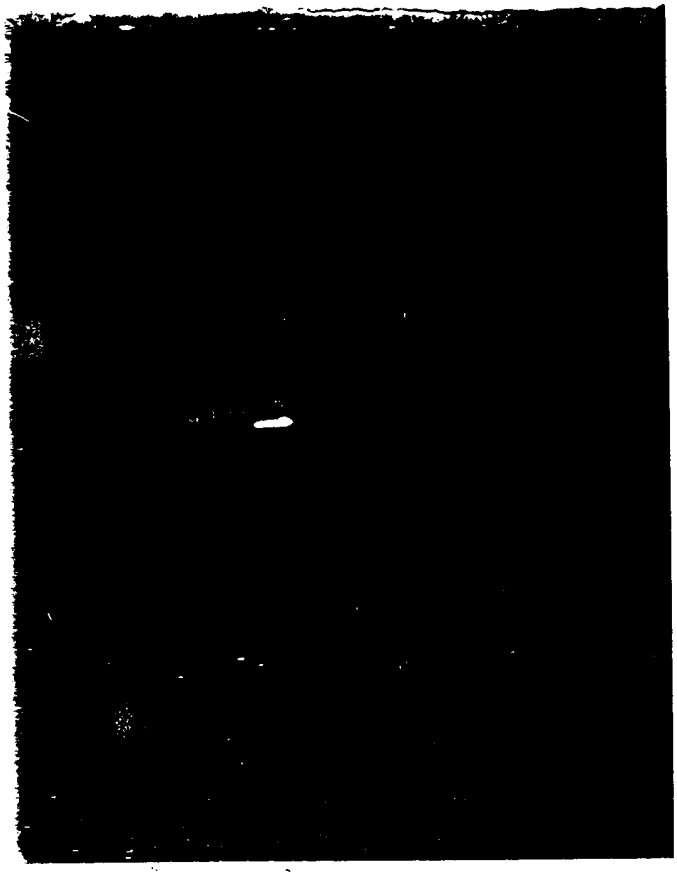
A $\frac{11,106}{140,868(2)}$ (1931) = 76

C $\frac{32,122}{39858(2)}$ (6,435) = 286

G $\frac{15,440}{153,372(2)}$ (8,336) = 420

T $\frac{5,142}{141,982(2)}$ (20,592) = 373

	#	%
A	76	6.6%
C	286	24.8%
G	420	36%
T	373	
total	1155	32.3% / 100.1%



A = .065 C = .248 G = .364 T = .323

term = 1.664903E-02
ala = 9.027199E-02
arg = .1004221
asn = 2.412475E-03
asp = 1.350986E-02
cys = 6.713361E-02
gln = 6.91548E-03
glu = 1.015014E-02
gly = .132496
his = 9.20452E-03
ile = 1.335282E-02
leu = .1248611
lys = 1.812525E-03
met = 7.64218E-03
phe = 5.957186E-02
pro = .061504
ser = 9.361386E-02
thr = .01612
trp = 4.279621E-02
tyr = 1.198815E-02
val = .117572

#1 eluted in ~ 11CV
#2 eluted in ~ 8CV

Sample

λ(nm)	1	2
260	.104	.269

← 1/100 dilution (2λ in 198λ TE

280	.054	.160

320

$$\frac{40X}{22} = \frac{mg}{ml} \quad (A_{260})$$

$$1 \Rightarrow \frac{40(.104)}{22}(100) = 18.9 \frac{mg}{ml} = \frac{\mu g}{\mu l} \quad (\text{dil. factor})$$

$$2 \Rightarrow \frac{40(.269)}{22}(100) = 49 \frac{mg}{ml} = \frac{\mu g}{\mu l}$$

Since these solutions are visibly darker
⇒ re-prep/dilute

dilute to total volume of 200λ for both 1 & 2

After 2nd phenol extraction/EtOH ppt:

	1	2
260	.60	.49
280	.28	.23
320	ϕ	.04

1:200 dil

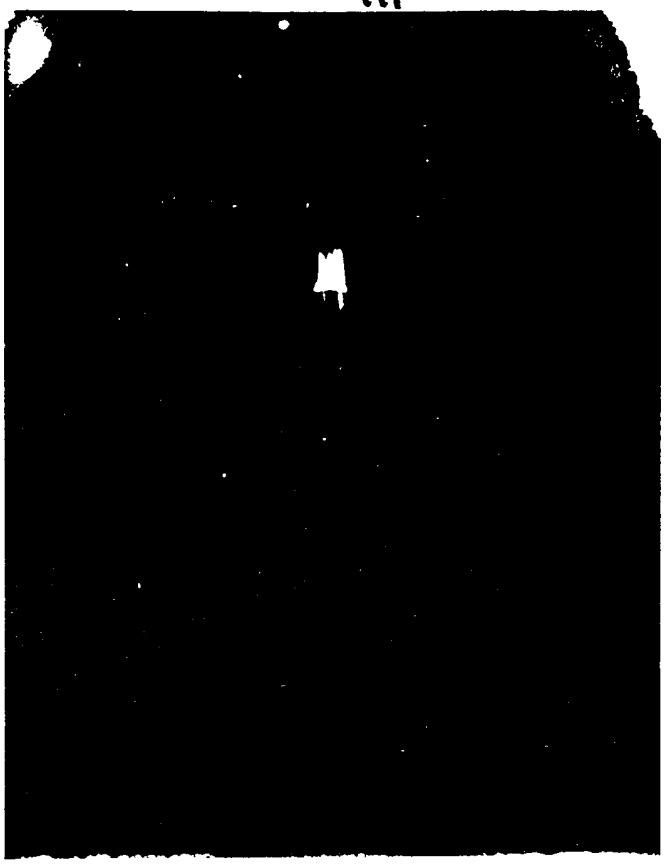
X = 260 reading

[1] ⇒ $\frac{200(.60)}{22} = 5.5 \frac{mg}{ml} = \frac{\mu g}{\lambda}$ (165 μg total in 30λ)

[2] ⇒ $\frac{20(.49)}{22} = 4.5 \frac{mg}{ml} = \frac{\mu g}{\lambda}$ (135 μg total in 30λ)

above in a total volume of ~30λ per 1 ml per L

1 2 13/14 III



Following 2 phenol/EtOH treatments (should be same as on p. 37 white, but obviously isn't!)

Puc19 check of concentration

260	.353
280	.183
320	0

dilution 1:100

$\frac{100(.353)}{22} = 1.6\ \mu g/\lambda$ (~65λ total)

Digestion of 19 w/ HindIII

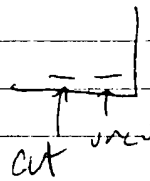
Set aside 5λ uncut in a separate vial in original DNA bearing vial (9μg)

60λ DNA @ 1.6 μg/λ (= 96 μg)
50λ HindIII @ 10U/λ (= 500 units : 2.5μg of BRL reddish BRL)
12.2λ 10X core buffer
122λ

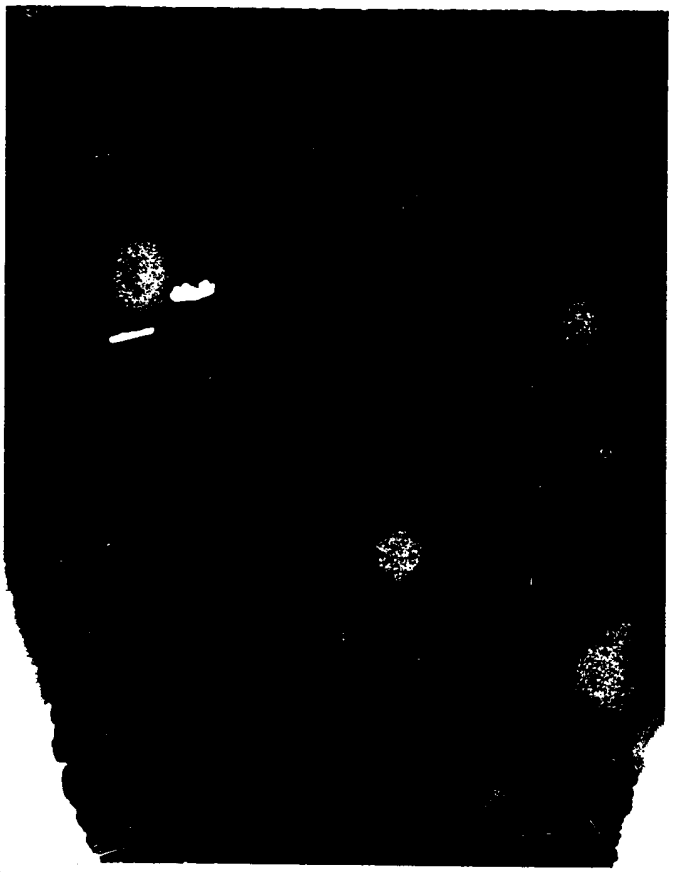
incubated at 37°C

@ 10:10am

After 4½ hours an aliquot (0.5λ) was run out on a gel



Puc 19
19/HIII



after
4 hrs ⇒

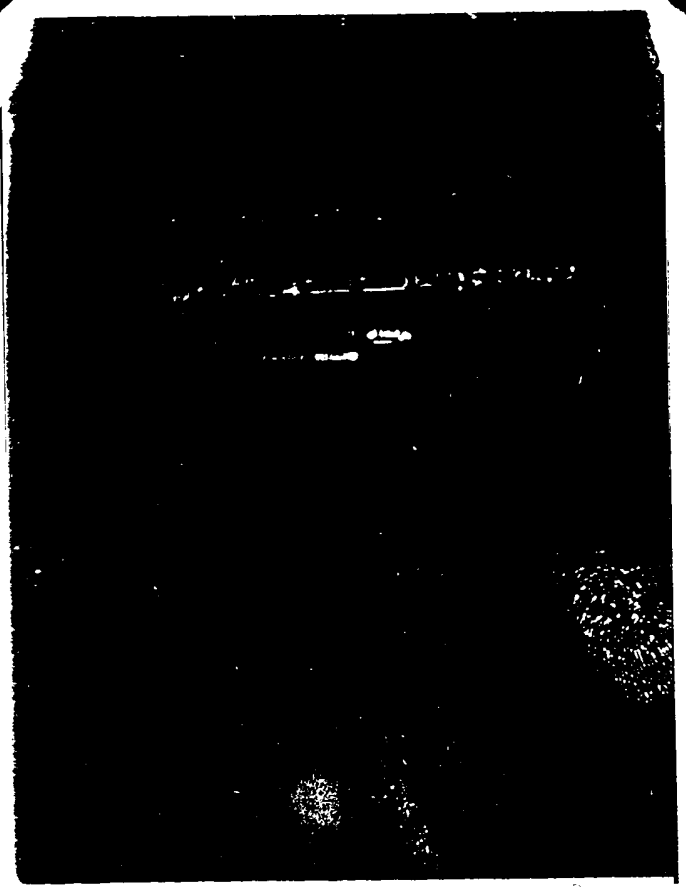
RXN allowed to continue for 10 hours then phenol/EtOH'd and reeluted in total of 600 TE (some spilled)

13-uncut
14-uncut
19/III-

	Puc19/1HIII
260	.118
280	.053
320	0

1:200 dil

$\frac{100(.118)}{} = .54 \mu g/\lambda$



~~The sect is should be ~3x as concentrated, but it does not look it. It's~~
~3x as much cut added as sent

TdT Random Tailing Pilot Rxn w/ PUC19

(similar to p.34 yellow)

✓ 1.	5λ pool ++ (5x)	St'd
✓	1.3λ PUC19/H^{III} at .54μg/λ	.7μg
	1λ $^{\alpha-32}$PdTTP	
	2λ ^{3}HdGTP	
	1λ 1:2.5 dil of TdT	
✓	14.7λ H_2O	
	25λ total	

2. Same as above, but

1λ $^{\alpha-32}$PdCTP

2λ ^{3}HdATP

- Before Adding TdT Spot 1λ of the rxn mixture
- After Rxn is over withdraw 5λ and place in separate culture tubes - these will be counted - the remaining 19λ should be chilled (& stored for purification methods)

10:45 am → 11:30 am
⇒ 45 minutes

Unfortunately, I forgot to change the filter between 1 & 2, so both counts were combined onto a single filter. About the only thing I can conclude is that the rxn worked

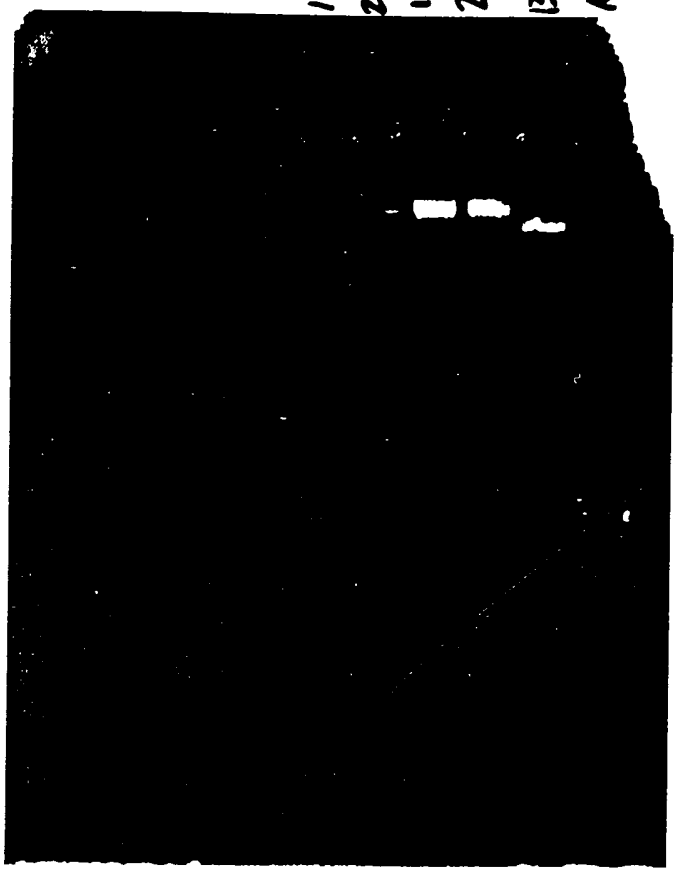
[redacted]

101° T=000.62 A=056764.5(1.5%) B=099917.7(1.0%) C=191385.4(2.0%) [illegible]
101° T=000.20 A=052200.0(2.0%) B=065355.0(2.0%) C=143760.0(2.0%) [illegible]
220 T=000.20 A=062800.0(2.0%) B=153300.0(1.5%) C=230320.0(1.5%) [illegible]

[redacted]

Of the 19λ remaining, 3λ each was saved (for an unpurified gel) and the other 16λ was phenol extracted/~~EtOH~~ ppt'd and re-eluted in 12λ each (per lane)

TE



~2X as much ethanol DNA sample as non-extracted sample added (5 even less concentrated relative to non-extracted from gel would suggest)

TdT purification Scheme Trial

Goal: To make TdT to test various methods of purification following IEX

1.	pool ++ (5λ)	std'd
~~7λ~~	pUC13/HIII at 0.5μg/λ	3.5μg
1.5λ	TdT	
1λ	α-32P dTTP	
~~45.5λ~~	H_2O	
10λ	total	

↑ incubate at 37°C for ½ hour

This should have been 125λ total ⇒ bases more concentrated (should not make too much of a diff since this exp. is aimed at separating labeled plasmid from dNTPs and Entire exp. is not to be measured after from visualization on a gel

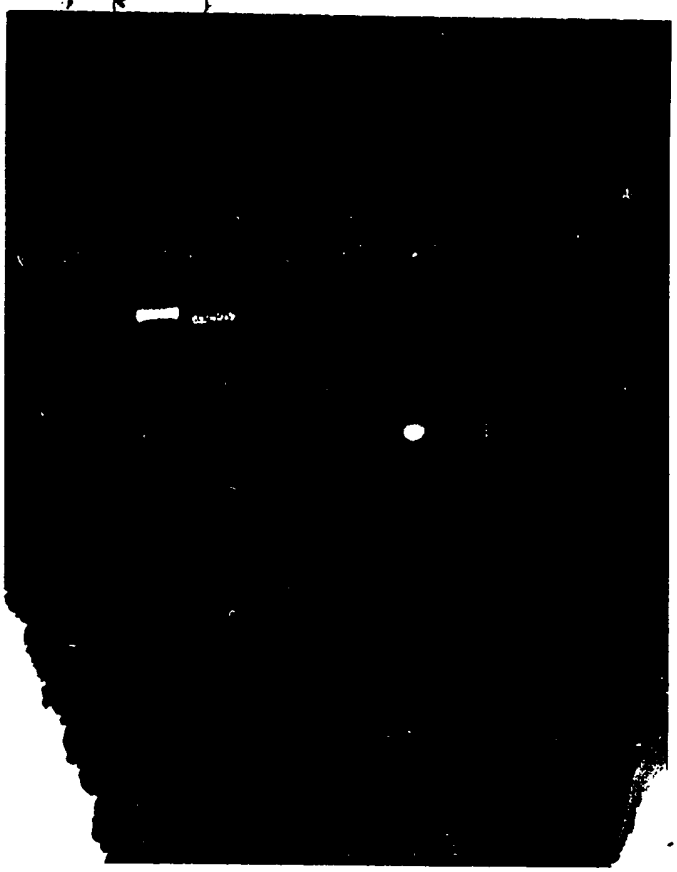
25λ withdrawn & worked up as regular BRL prep

↓

After EtOH ppt resulted in 10λ TE – 6λ of this run on gel w/

- 6λ Nacs purified 13/III trial (25λ → Nacs → 10λ ↑ 6 this
- 6λ raw IEX
- 1λ pUC13/HIII

Naes Tris-Ac pH 7.5



Mucho the "Naes" floated away upon loading because there was still plenty of EtOH not yet dryed from the pellets. (And there was a low ratio of loading buffer)

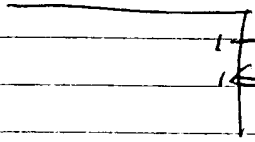
The "Naes" was completely cold upon loading ⇒ all the dNTP's were gone (By contrast the phenol/EtOH samples are still pretty hot ⇒ dNTP's present)

Preparation of NAES

1 gram of Nacs 52
10 ml 2M Nacl TE

- mix gently for 1 hour
- allow matrix to settle for 1 hour - remove SN by asp
- repeat until SN is clear (1 more time)
- store at 4°C until

25 ul of the previous days sample was loaded in a 1cc syringe nax column, eluted in 4 ~~ml~~ ppt, redissolved in 25 ul. 6M GuSCN loading buffer) of the Nax was compared at an equal amt (& equiv. concentration) on a gel



↳ gel ⇒ DNA recovered, at least none appeared on gel (no bottom for the picture)

Large Scale Random Tail of PUC 19

42.3λ scaled

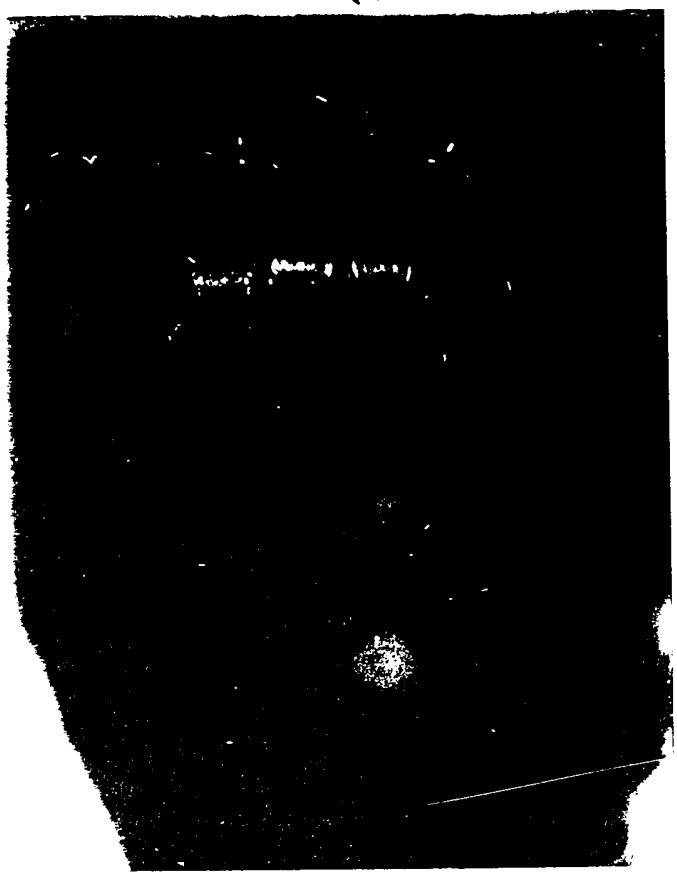
55λ PUC19/H^{III} at .54μg/λ — 29.7μg
212λ 5X pool^{++} — std
16.9λ TdT
— clavulo
— H_2O
1058λ total

1. 27.5λ PUC19/H^{III} at .54μg/λ — 14.9μg
106λ 5X pool^{++} — std
8.5λ TdT
5λ $^{\alpha-32}$P dTTP
10λ ^{3}H dGTP
372λ H_2O
total
529λ

2. Same as above but
5λ $^{\alpha-32}$P dCTP
10λ ^{3}H dATP
Incu at 37°

- Before Adding TdT withdraw 5λ from each tube & spot as the pool
- After rxn ends (1 hour) Withdraw 10λ from each tube to save for Counting
- The rest is gel loaded for purif.

13/III



193 T=000.50 A=017552.0(3.0%) B=004162.0(5.0%) C=[illegible]

194 T=000.50 A=007704.0(5.0%) B=029448.0(2.0%) C=043361.0

195 T=000.50 A=120550.0(1.0%) B=087418.0(1.0%) C=257[illegible]

196 T=000.50 A=063906.0(1.5%) B=290984.0(0.7%) C=451865.0[illegible]

[A] $\frac{.7704}{63906(2)}(1931) = 116$

[C] $\frac{29,448}{290,984(2)}(6,435) = 326$

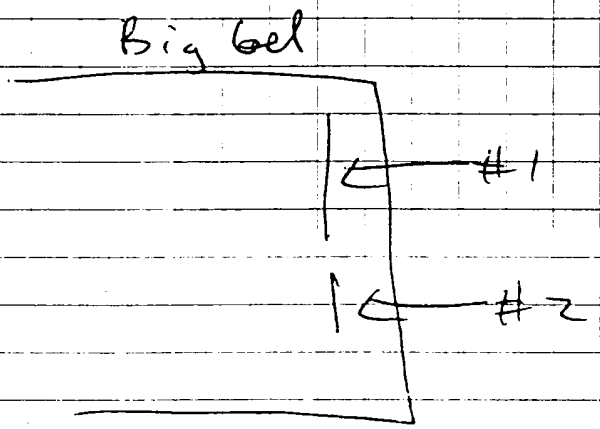
[G] $\frac{17,552}{120,550(2)}(8,336) = 607$

color [T] $\frac{4162}{87418(2)}(20592) = 490$

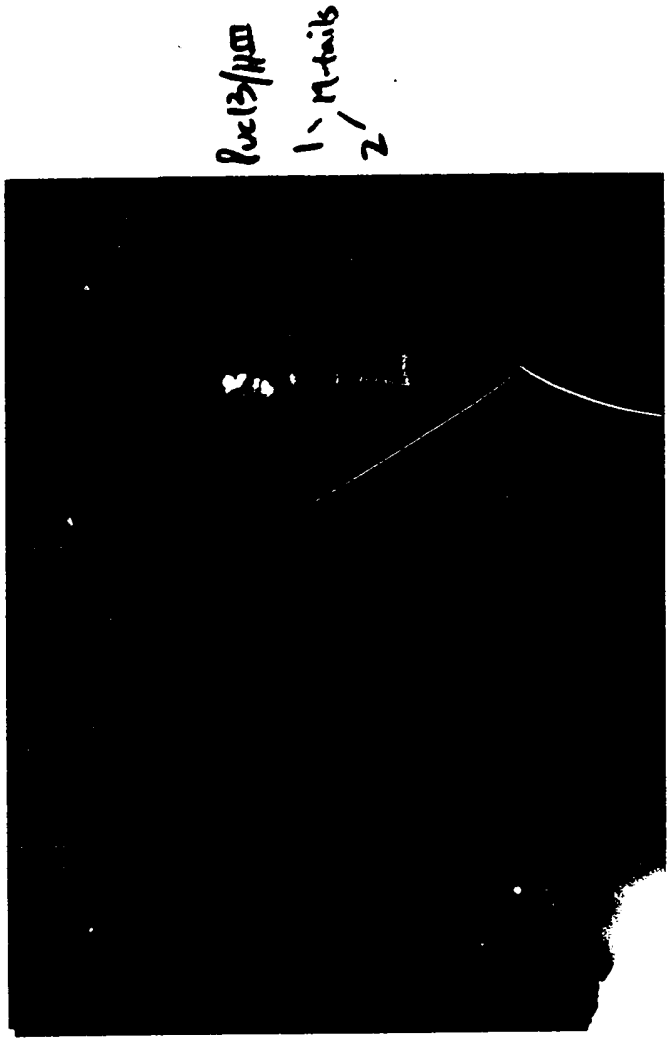
	#	%
A	116	7.5%
C	326	21.1%
G	607	39.4%
T	490	31.8%
	1539	99.8

A = .075 C = .212 G = .395 T = .318

term = 2.063025E-02
ala = 8.374001E-02
arg = 9.766374E-02
asn = 2.98125E-03
asp = 1.570125E-02
cys = .0665733
gln = 7.473001E-03
glu = 1.392375E-02
gly = .156025
his = 8.426999E-03
ile = 1.442925E-02
leu = .1149443
lys = 2.64375E-03
met = 9.42075E-03
phe = 5.359572E-02
pro = .044944
ser = 8.311725E-02
thr = .0159
trp = 4.961595E-02
tyr = .0126405
val = .12561



Purified according to Ray's Protocol, #1 & #2
each eluted in ~130λ TE



λnm	1	2
260	.165	.150
280	.122	.100
320	.036	.017

1:40 dilution

1 ⇒ $\frac{40(.165)}{22} = .3\,\mu g/\lambda$

another way to do calc.

1,552(52.9) = 928,500
total counts putin

1833(80) = 146,640

293,280 ⇒ 1/3 counts
928,500 received

146,640 ⇒ ~16%
928,500

2λ from both 1&2 was dotted on filter, dried & counted

[redacted]

^{3}H ^{32}P

T=001.00 A=001533.0(5.0%) B=000399.0(1.5%) C=002600.0(5.0%) P=0[illegible]

T=001.00 A=001523.0(7.0%) B=004520.0(3.0%) C=007654.0(3.0%) S=[illegible]

2λ counted

[redacted]

From counts taken on sample following taking 1λH

1λ counted $^{3}H \Rightarrow \frac{17,552 \text{ cpm}}{10\lambda} = \frac{1,755 \text{ cpm}}{\lambda}$.

The above $\Rightarrow \frac{1,833 \text{ cpm}}{2\lambda} = \frac{917 \text{ cpm}}{\lambda}$

~~1,755~~

$\frac{917}{1,755} = .52$ x as concentrated

$^{32}P \Rightarrow \frac{4162 \text{ cpm}}{10\lambda} = \frac{416 \text{ cpm}}{\lambda}$

$\frac{400 \text{ cpm}}{2\lambda} = \frac{200 \text{ cpm}}{\lambda}$

$\frac{200}{416} \cong .5$ x as concentrated as original sample

orig. sample had $\frac{14.9\,\mu g}{527} = \frac{.028\,\mu g}{\lambda}$

filtered aqueous extraction $= \frac{.025\,\mu g}{2\lambda} = \frac{.014\,\mu g}{\lambda}$

(1 λ contains 2.24 μg total)

---

(2) ^3H: $\frac{7,704\ cpm}{10\lambda} = \frac{770\ cpm}{\lambda}$

$\frac{1543\ cpm}{2\lambda} = \frac{771\ cpm}{\lambda}$

$\frac{771}{770} \approx$ the same concentration as original sample

^{32}P: $\frac{29,448\ cpm}{10\lambda} = \frac{2,944\ cpm}{\lambda}$

$\frac{5329\ cpm}{2\lambda} = \frac{2,665\ cpm}{\lambda}$

(3) about the same concentration as original PAH MIX

orig concentration:

$\frac{14.9\mu g}{529\lambda} = \frac{.028\mu g}{\lambda}$

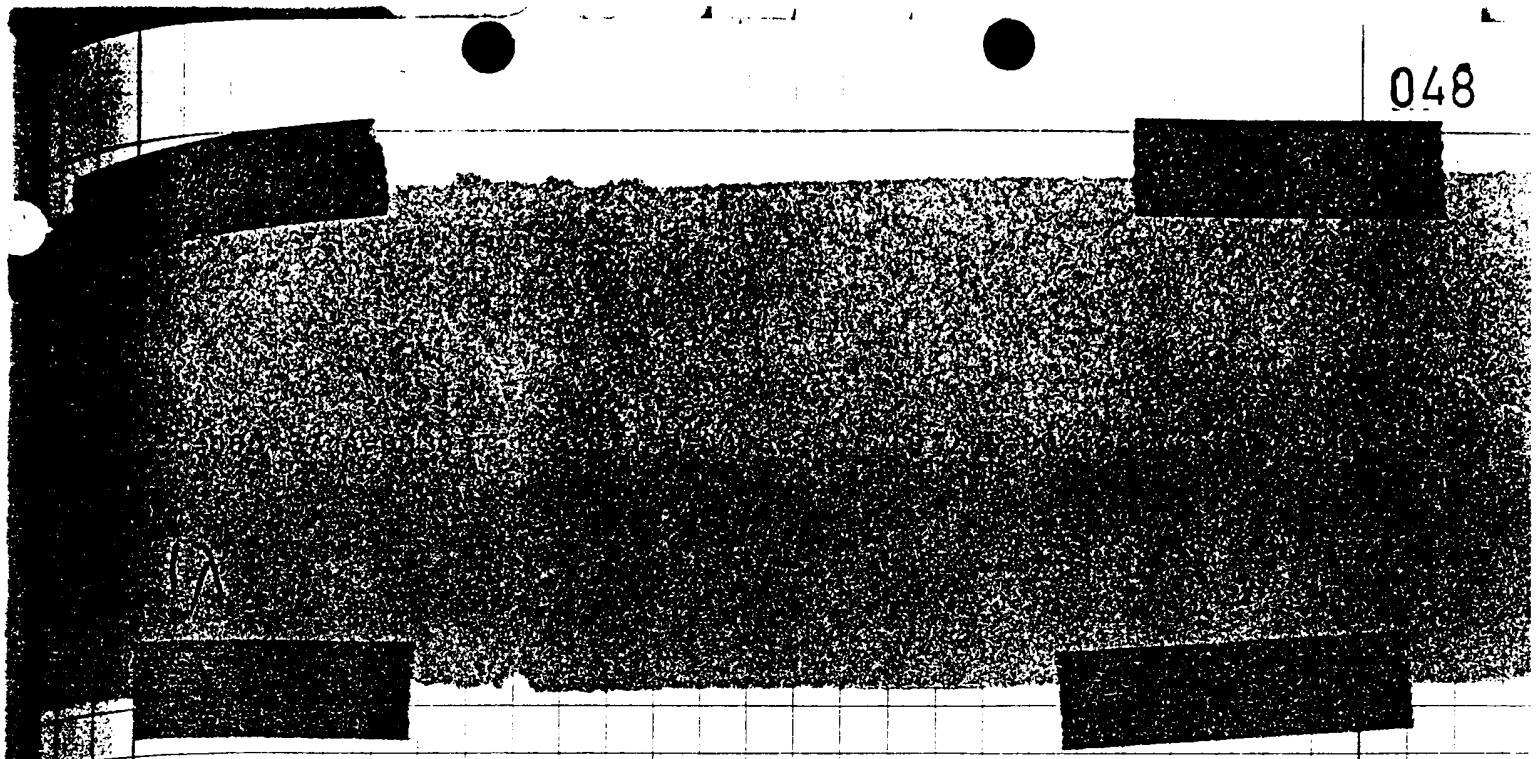
So, purified DNA ~ $.028\frac{\mu g}{\lambda}$

(160λ ⇒ 4.5μg total)

The DNA was once again phenol extracted & EtOH ppt
— each 1&2 was redissolved in 20λ TE

~~It was dried on filter & counted~~

^3H ^{32}P



New Puc 19

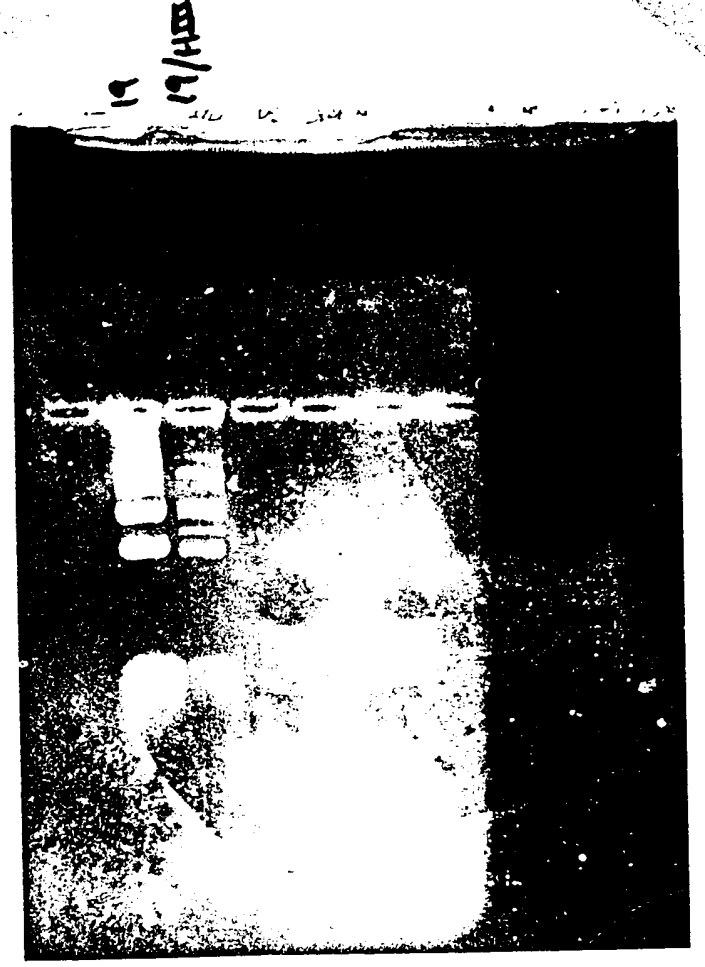
~~Redo Puc 19 @ 13.07 mg/ml~~

~~Redo Puc 19 @ 13.07 mg/ml~~

26.7 λ Puc 19 DNA @ 13.07 mg/ml (= 349 µg)
100 λ ~~Bam~~ HindIII @ 10 U/λ (= 1,000 units)
14 λ 10X cut buffer
140.7 λ total

37°C @ 9:30 pm

← after 12 hours



To the above rxn mix:

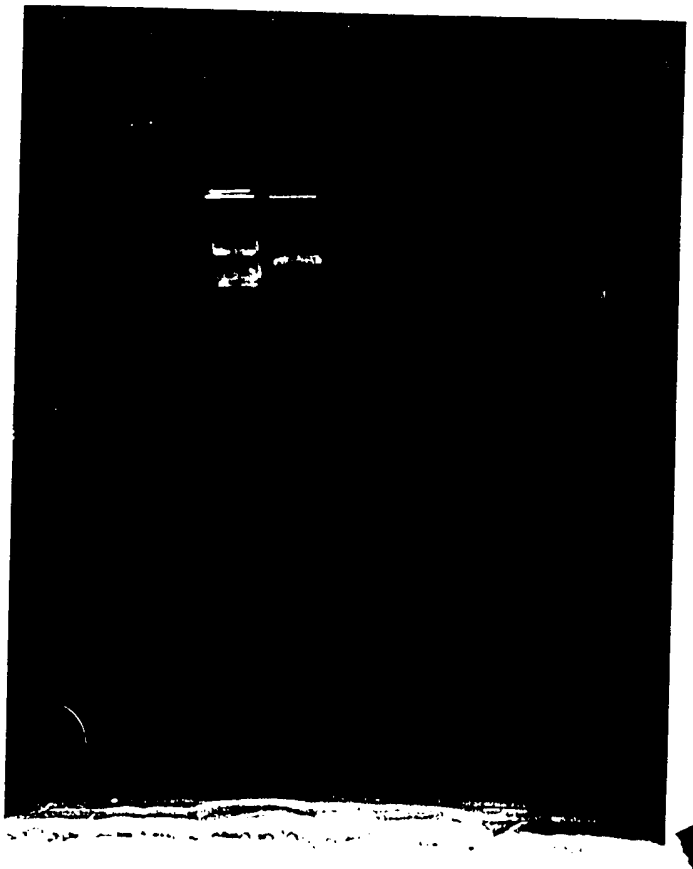
existing
- 26.7 λ PvC19 @ 13.02 mg/ml (349 μg)
- 100 λ HindIII at 10 u/λ (1,000 units)
- 14 λ 10× core buffer
- 140.7 λ total

- 150 λ H_2O
- 50 λ HindIII
- 23.9 λ 10× core buffer

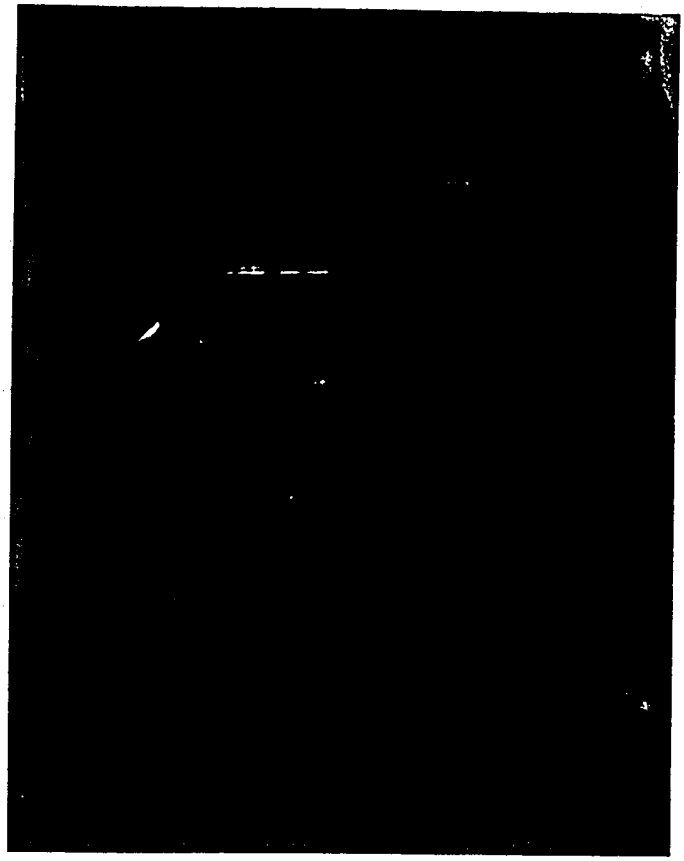
new total 364.6 λ
+ 180 λ H_2O
+ 20 λ 10× core

564.6 λ total @ 11 am 7-25-84

Shows following dilution & addition of core enzyme



Dec
9am on



15λ of Lyph. (reconst.) HIII was added to 9am mix (15λ · 9μ/λ) = 135μ

2

Placed into 2 tubes

tube 1	tube 2	tube 3
~2407	~2407	

The mixture was EtOH, EtOH/$CHCl_3$, and $CHCl_3$ extracted but not ppt'd. The organic phase was pooled and "back extracted"

nm		"back extracted"
260	.500	.305
280	.278	.197
320	.001	.004

diluted 1:40

$\Downarrow$

$\frac{40x}{22}$

Concentration $\frac{40(.5)}{22} =$

$= \boxed{.91 \mu g/\lambda}$

(~200λ ⇒ 182μg)

$\frac{260}{280}$ ratio 1.8

$\frac{40(.305)}{22} = \boxed{.55 \mu g/\lambda}$

(~220λ ⇒ 121μg

1.5

TdT Assay

Goal: To check activity of P-L TdT
(based on assay on p. 41 in unit)

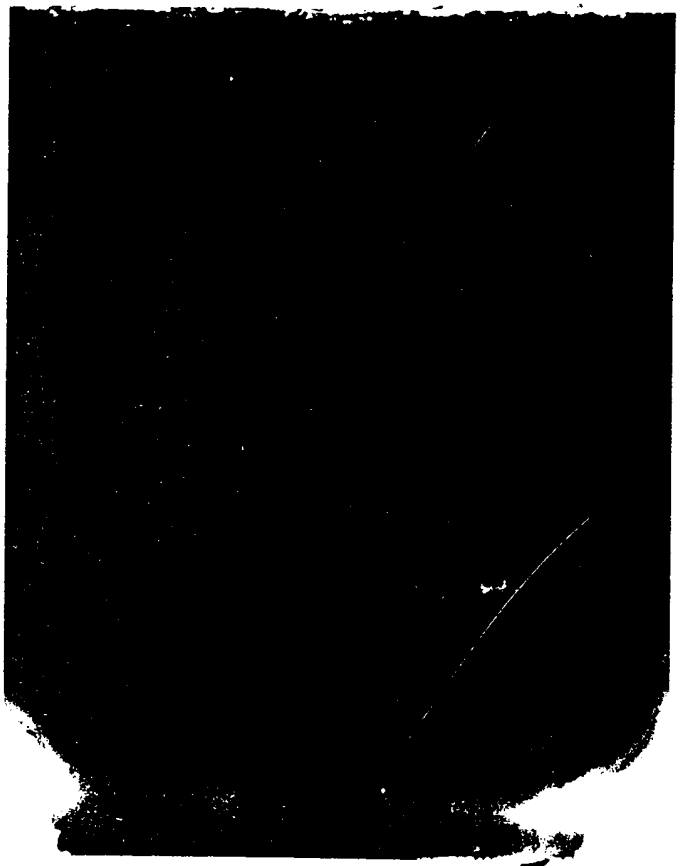
✓ 5λ pool++ (5X) 5 t'd
✓ 0.8λ ddNTP/Fluorescein-dUTP @ .9 μg/λ .72 μg
2.5λ P-L TdT
1λ $^{\alpha-32}$P dGTP
2λ ^{3}H dGTP
13.7λ H_2O
25λ TdTed

Same as above except
1λ $^{\alpha-32}$P dCTP
2λ ^{3}H dATP

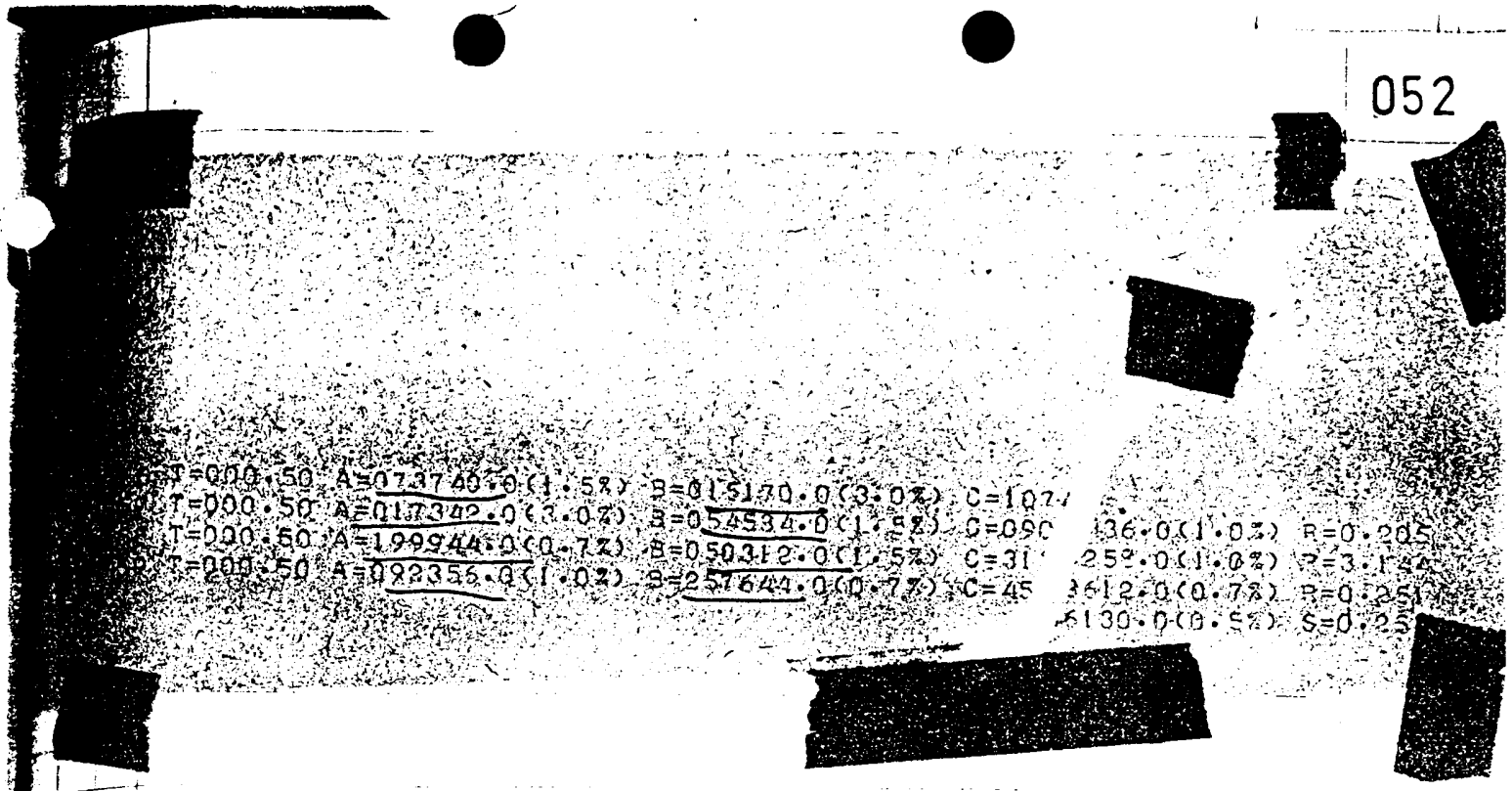
~~Exc eqn~~

~~Exc~~

Before adding TdT 2λ from each is spotted
After rxn 6λ from each is withdrawn & placed on a gel



M-54



[A] $\frac{17,342}{92,356}\frac{(2)}{(17)}(1931) = 43$

[C] $\frac{54,534}{257,644}\frac{(2)}{(17)}(6,435) = 160$

[G] $\frac{73,740(2)}{199,944(17)}(8,336) = 362$

[T] $\frac{15,170(2)}{50,312(17)}(20,592) = 730$

	#	%	
A	43	3.3%	$\Rightarrow$ 1.87
C	160	12.4%	
G	362	28.0%	
T	73?	<1.4	

. quit A = .033 C = .123 G = .28 T = .564
*** END RUN dBASE II ***

term = 1.1036923-02
ala = .03444
arg = 3.7332127-02
asn = 7.431437-04
asp = 6.347035-03
cys = .1084911
gln = 1.2704677-03
glu = 2.092127- 3
gly = .3784
his = 2.7885337-03
ile = 1.3400647-03
leu = .1689361
lys = 3.408573-04
met = 5.2113617-03
phe = .218532
pro = .215129
ser = 7.5719037-03
thr = .204059
trp = .0442171
tyr = 1.2706437- 3
val = .15792

A new batch of pool++ was prepared according to recipe on p.32ii

TdT Assay: To test out new pool in anticipation of yet another large scale tailing rxn

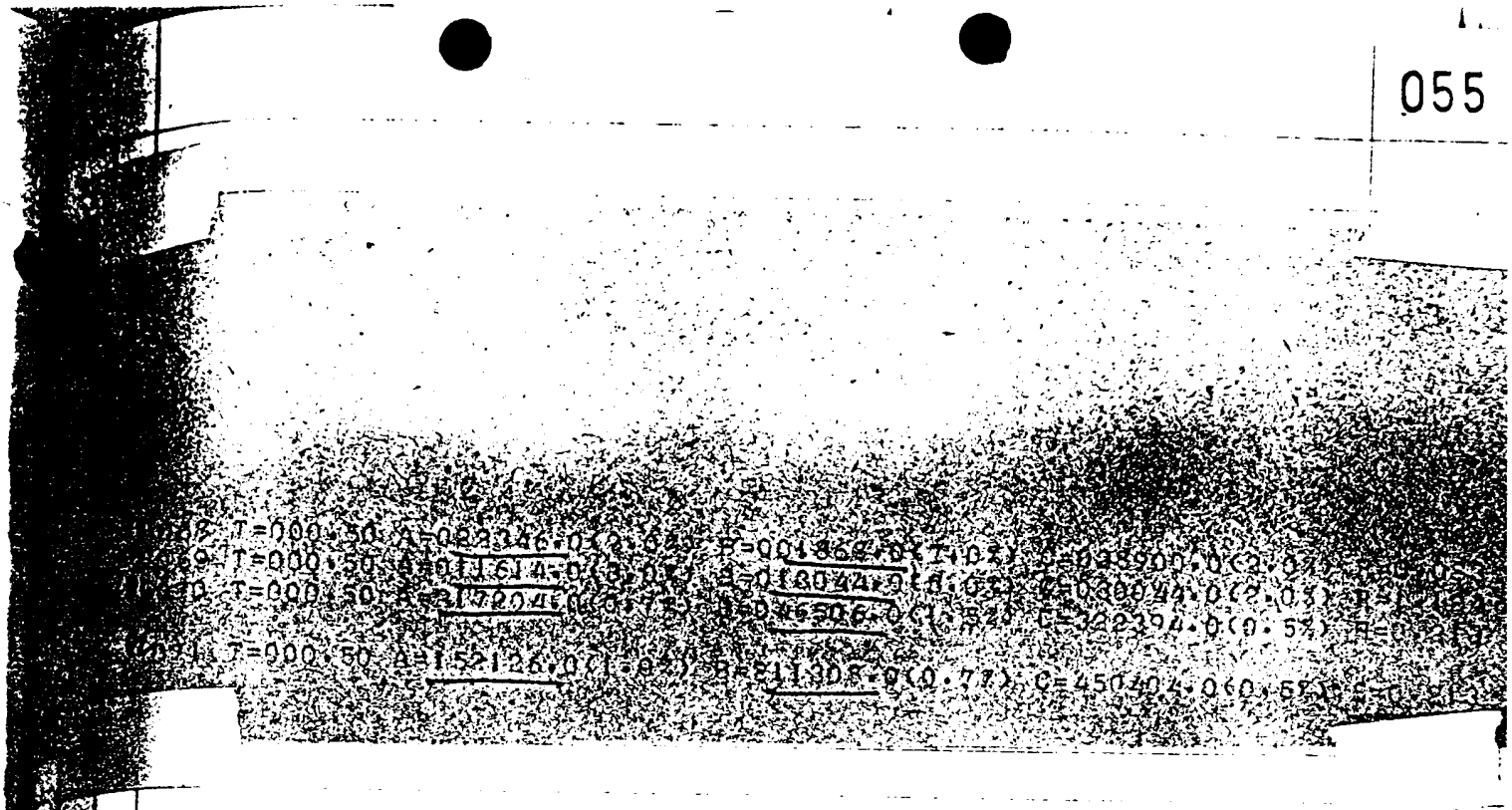
① 5λ	pool++ (5X)	St'd
0.8λ	Puc 19/HindIII @ .91 μg/λ	.72 μg
1.8λ	P-L TdT	
1λ	α-32P dCTP	
2λ	3H dGTP	
13.2λ	H_2O	
25λ	total	

② as above except
1λ α-32P dCTP
2λ 3H dATP

rxn went 45 mins.

Before adding TdT spot 2λ
After rxn 2λ withdrawn for gel

run out on a gel - looked as expected
not photographed



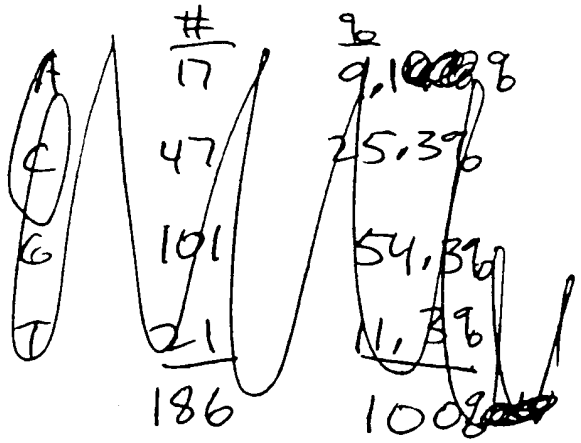
A $\frac{11,614\ (2)}{152,126\ (17)}\ (1931) = 17$

C $\frac{13,044\ (2)}{211,308\ (17)}\ (6,435) = 47$

G $\frac{22,346\ (2)}{217,204\ (17)}\ (8,336) = 101$

T $\frac{1,868\ (2)}{46,506\ (17)}\ (20,592) =$ 97.3

	#	%
A	17	6.5%
C	47	17.9%
G	101	38.5%
T	97.3	37.1%
	262	100%



TdT Assay

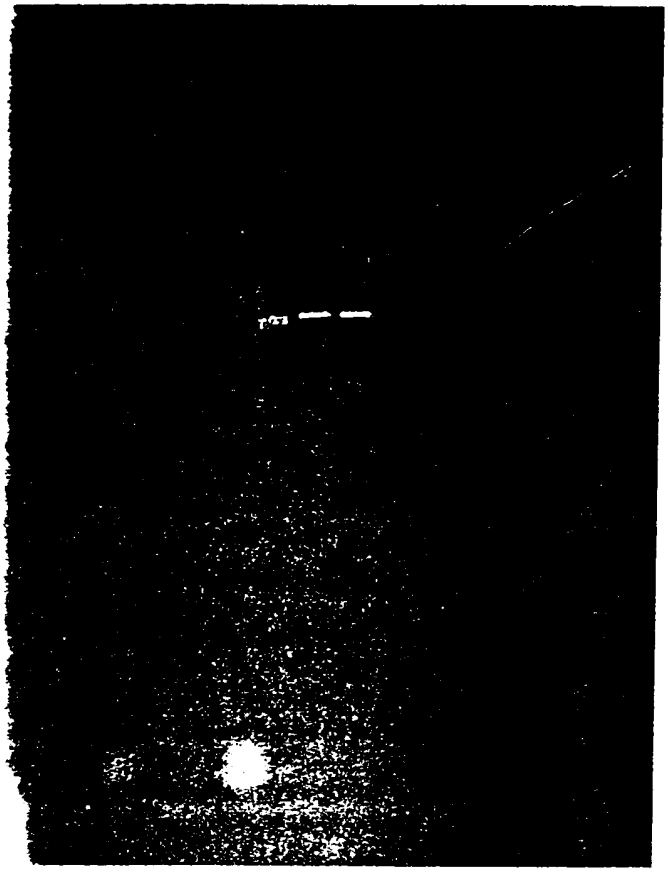
This assay uses the old "batch 2" of TdT (see p. 3W7)

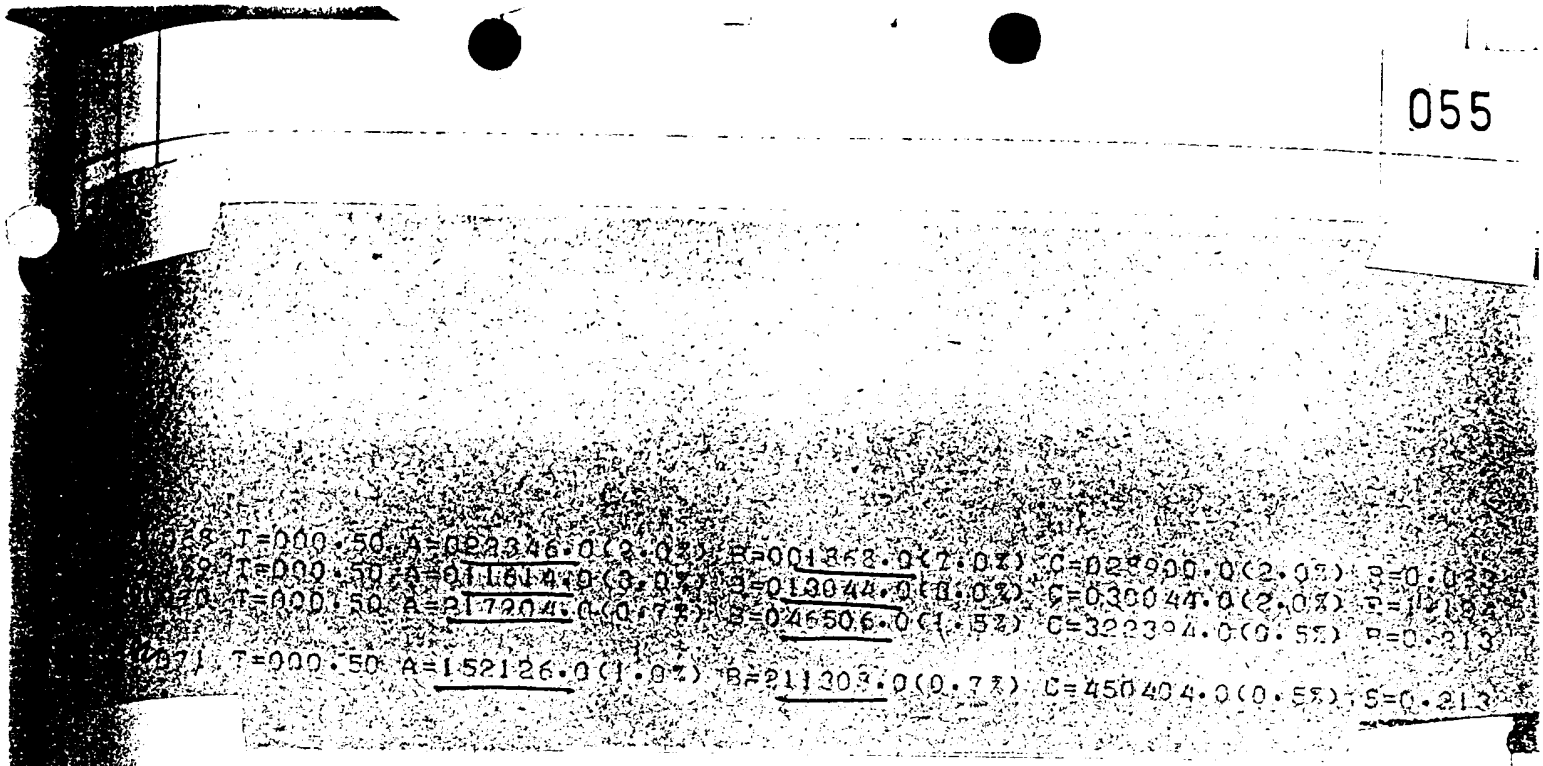
1.	5λ	pool ++ (5X)	std
	0.8λ	pUC19/HIII @ .91μg/λ	.72μg
	1.5λ	"batch 2" TdT	
	1λ	α-32P dTTP	
	2λ	3H dGTP	
	14.7λ	H_2O	
	25λ	total	

2. Same as above except
   - 1λ α-32P dCTP
   - 2λ 3H dATP

- Before adding TdT spot 2λ
- After 1 hr incubate cathodrons @ for gel check

~~1-10 min~~ 45 min





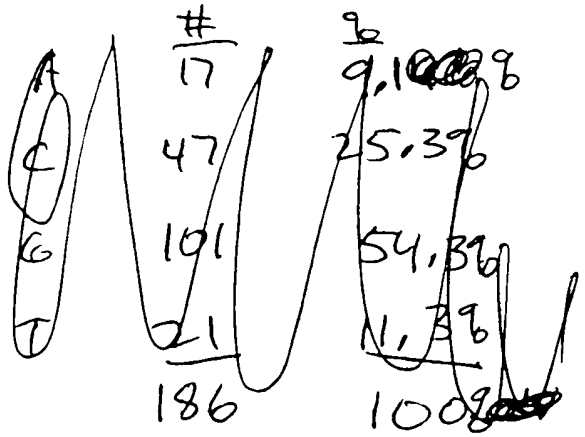
A) $\frac{11,614\ (2)}{152,126\ (17)}\ (1931) = 17$

C) $\frac{13,044\ (2)}{211,308\ (17)}\ (6,435) = 47$

G) $\frac{22,346\ (2)}{217,204\ (17)}\ (8,336) = 101$

T) $\frac{1,868\ (2)}{46,506\ (17)}\ (20,592) = 97.3$

	#	%
A	17	6.5%
C	47	17.9%
G	101	38.5%
T	97.3	37.1%
	262	100%



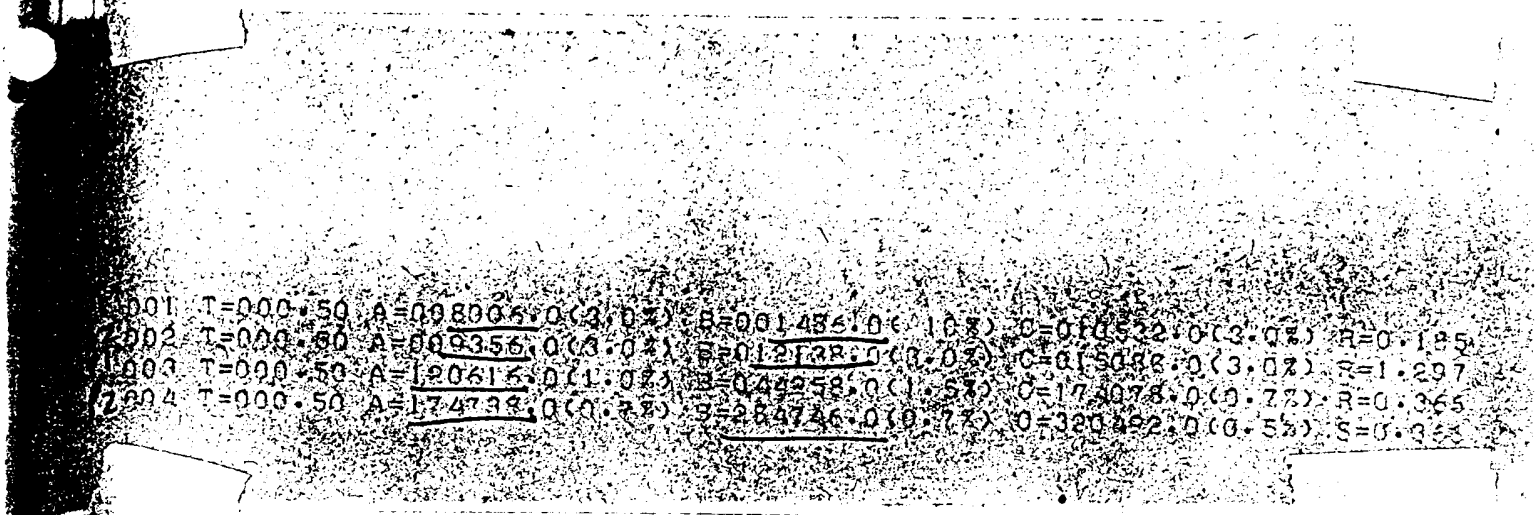
TdT Assay: To find the proper amt of batch 2

		std
1. 5λ	pool ++ (5X-batch 2)	
0.8λ	pUC19/HindIII @ .9/μg/λ	.72μg
6.5λ	"batch 2" TdT	
1λ	α-32PdTTP	
2λ	3HdGTP	
9.7λ	H_2O	
25λ	total	

2. same as above except
1λ α-32PdCTP
2λ 3HdATP

-before adding TdT spot 2λ
~~After 15m reminder was drawn for getches~~

~~1 hr~~ 40 minutes



[A] $\frac{9356}{174{,}738(11.5)}(1931) = 9$

[C] $\frac{12138}{284{,}746(11.5)}(6{,}435) = 24$

[G] $\frac{8006}{120{,}616(11.5)}(8336) = 48$

[T] $\frac{1486}{44{,}258(11.5)}(20{,}592) = 60$

	#	%
A	9	6.4%
C	24	17.0%
G	48	34%
T	60	42.6%
	141	100%

There appeared to be a lot of glycerol in rxn mixture — maybe so much so that the rxn was inhibited

TdT Assay

1. ✓ 5λ pool ++ (batch 2)
✓ 0.8λ PUC19/HIII @ .91 μg/λ .728 μg DNA
3λ "batch 2" TdT
1λ α-32PdTTP
2λ 3HdGTP
✓ ~~13.2λ~~ H_2O
~~25λ total~~ → actually 23λ total since 2λ withdrawn

2. Same as above except
1λ α-32PdCTP
2λ 3HdATP 45'

Before Adding TdT ~~withdrawn~~ 2λ
½λ withdrawn for gel
→ checked, not photographed — looked good — no detectable exo activity

~~3'OH~~ The calculation of the length is wrong. It depends on the concentration of DNA ~~strands~~ carrying in the original assay since per ~~3'OH~~ However, more DNA was added. Revised # ~~of~~ dNTPs/3'OH are below.

(See p. 32 y/bench)

~~5 ng/µl~~

~~$\Rightarrow (.05)(1.15\times10^{-12}$ mole 3'OH per µg)~~

5 µg $\Rightarrow$ $5(1.15\times10^{-12}$ 3'OH Moles / µg$)$

$= 6.325\times10^{-11}$ moles 3' ends

$\frac{6.325\times10^{-11} \text{ moles 3'OH}}{.0010125 \text{ l}}$

$= 5.9\times10^{-8}$ M

$.06\text{mM} = .06\times10^{-3}$ M

$= 6\times10^{-5}$ M

#A $\frac{6\times10^{-5}\text{ M dATP}}{5.9\times10^{-8}\text{ M 3'OH}} = 1{,}017$

#C $\frac{2\times10^{-4}\text{ M}}{5.9\times10^{-8}} = 3{,}390$

TdT Random Tailing - Large Scale

[illegible]

42.9 fold scaleup

1. 60.5λ Puc 19/HIII @ .9 μg/λ — 55 μg DNA
   215λ PdeTT
   129λ TdT - "batch 2" (@ 100 μg/ml)
   6λ α-32 PdCTP
   10λ 3HdGTP
   652λ H_2O
   1072.5 total

2. Same as above except
   6λ α-32 PdCTP
   10λ 3HdATP

- Before adding TdT withdraw 5λ and spot from each tube
- At conclusion of rxn withdraw from each tube
  - 10λ for counting - into culture tube
  - 6λ for mini gel

410 minutes

Note - [illegible] was withdrawn before the [illegible] quantity of TdT was added ⇒

$$\frac{107.5 - x}{107.5} = \frac{}{}$$

$$\frac{107.5}{107.5 - 5.29} = 1.14 \times \text{as concentrated}$$

hence $5\lambda(1.14) = 5.68\lambda$ of pol equiv. drawn

T=000.50 A=003318.0(5.0%) B=000202.0(.20%) C=004160.0(5.0%) T=0.0[illegible]
T=000.50 A=001248.0(1.0%) B=002008.0(2.0%) C=002135.0(1.5%) S=1.[illegible]

T=000.50 A=032962.0(1.0%) B=023352.0(2.0%) C=111408.0(1.0%) S=0.[illegible]
T=000.50 A=036228.0(2.0%) B=076976.0(1.5%) C=074308.0(1.5%) S=0.[illegible]

[A] $\frac{1248\ (5.68)}{36228\ (10)}(1931) = 38$ .0195

[C] $\frac{2008\ (5.68)}{76976\ (10)}(6,435) = 95$ .0148

[G] $\frac{3318\ (5.68)}{82962\ (10)}(8,336) = 189$ .0227

[T] $\frac{302\ (5.68)}{23352\ (10)}(20,592) = 151$ .00735

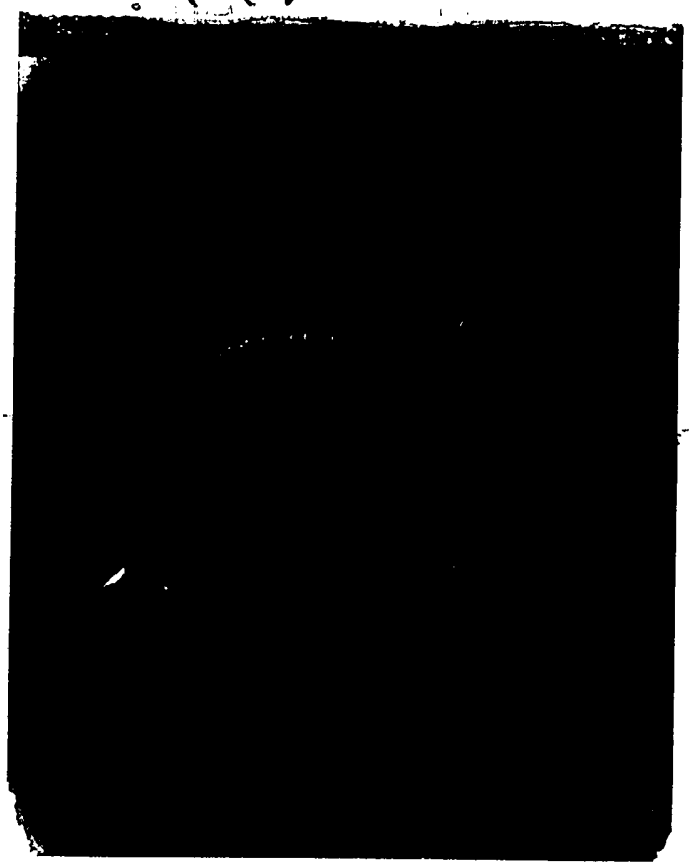
A	38	8.0%
C	95	20.0%
G	189	40.0%
T	151	32.0%
	~~473~~	100

249

1-87

p(TAA) (.08)(.32)(.32) = .00819
p(TAG)
p(TGA)) = (.32)(.08)(.40)
= .01024
= .09432

$(98.1568)^{100} \Rightarrow$ 15.6%



I'm somewhat skeptical of the counting results ~~the gel shows~~ adequate)
Stable isolate

~~5 mg~~ withdrawn & phenol extracted
LL phenol, 1x LL phenol/chloroform, chloroform → ether

#1 phenol'd & checked on 9 µgel = looked good
So #2 also phenol'd, but not checked
both #1 & #2 need to let ether further evaporate from them

~ 1/2 of #1 loaded into Naes
(~ 27.5 µg DNA)
→ 20λ / diluted to 5.2 ml w/ binding buffer

eluted in ~~250 µl 3x25 Naes~~
825 µl 3x275
EtOH ppt

pellet size removed quite small

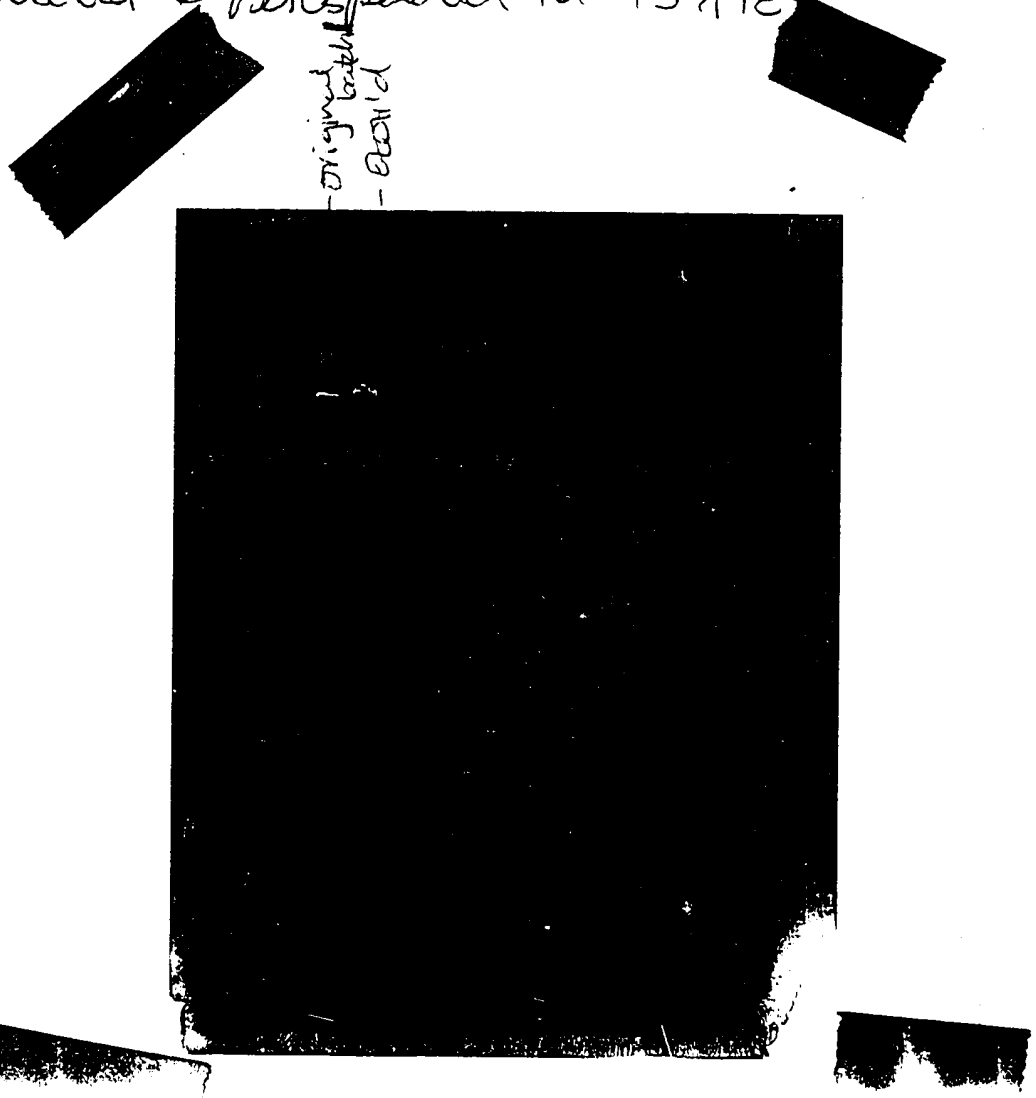
Attempted Purification scheme: EtOH ppt - but first chelate w/ EDTA

100λ aq rxn #1
5.5λ aq 500mM EDTA
11λ 3M ~~Sodium~~* acetate
291λ 95% EtOH

5μg DNA in 1mM $CoCl_2$
26mM EDTA
283mM NH_4 acetate

* Note: Not NH_4!

pelleted & resuspended in 15λ TE



OD measurements

	raw rxn #1 (phenol'd)	EtOH'd #1
260	.439	.738
280	.279	.513
320	0	.057

1:40 dilution

Counting Experiment

"P1" = 3λ of #1 raw and 3λ of #1 EtOH'd were dropped on filters dried & counted

"P1E" =

"1" = 3λ of #1 raw and 3λ of #1 EtOH'd were acid ppt and filtered & counted according to the normal polymerase assay

"1E" =

[redacted]

1 refers to rxn mixture #1

^{32}P pool acid ppt: A: ^3H B: ^{32}P

```
095 T=000.50 A=0[illegible](1.5%) B=010928.0(3.0%) C=058[illegible].0(1.5%) R=0.245
C 096 T=000.50 A=138175.0(1.0%) B=021394.0(2.0%) C=174614.0(0.7%) R=0.140
097 T=000.50 A=001090.0( 10%) B=0000[illegible].0([illegible]10%) C=001424.0( 15%) R=0.055
E 098 T=000.50 A=004344.0(5.0%) B=000323.0( 30%) C=014704.0(3.0%) S=0.055
```

acid ppt: 3λ 1

(these vertical lines are here by mistake)

[redacted]

Interpretation & calculations from the above

I. First of all, these results (P1 & 1 should be in agreement with the earlier pool & acid ppt counts

A. p.58W ⇒

P1: ^3H $\begin{cases} \frac{3}{5}(82{,}962) \overset{?}{=} 44{,}566 \\ 49{,}777 \approx 44{,}566 \end{cases}$ ⇒ Counting of pool for rxn #1 good

^{32}P $\begin{cases} \frac{3}{5}(23{,}352) \overset{?}{=} (10{,}928) \\ 14{,}011 \approx 10{,}928 \end{cases}$ ↗

B.

1: ^3H $\{ .3(3318) \overset{?}{=} 1090$
$995 \approx 1090$ ⟹ Counting of acid ppt'ble counts for rxn #1 good

^{32}P $\{ .3(302) \overset{?}{=} 66$
$90.6 \approx 66$

III. Recovery on EtOH ppt

(Note the phenol extraction is assumed to have 100% – this should be tested)

For "1" 3λ of the crude mixture had 1090 ^{3}H
for "1E" 3λ had 4644 ^{3}H counts

but 1E represents 100λ of "1" resuspended in only 15λ TE

$\Downarrow$

$\frac{100}{15} = 6.7$ fold concentration

$\frac{4644}{1090(6.7)} = 64\%$ recovery

Based on ^{32}P counts

$\frac{328}{666(6.7)} = 74\%$ recovery

$\Rightarrow \sim 70\%$ recovery of DNA

IV. 60% dNTP removed by EtOH ppt!

^{3}H { counts due to dNTP alone
{ 44,566(6.7) − 1090(6.7) ← before EtOH ppt
{ 133,176 − 4644 ← after EtOH

^{3}H { counts due to dNTP alone ← after EtOH ppt
{ 133,176 − 4644 / (illegible) = .44 (illegible) due to (illegible) remaining

32p [illegible] ~~21394-328~~

$$^{32}P\ \{\ \frac{21394-328}{(10{,}928-66)(67)} = .29$$

$^{3}H \Rightarrow$ 56% of unincorporated dNTP removed

$^{32}P \Rightarrow$ 71% of unincorporated dNTP removed

Conclusions-

1. ~70% of the DNA was recovered by EtOH ppt. (concentration of DNA acceptable from this fact)
2. ~60% of the unincorporated dNTP was removed
3. A relatively small percentage of the counts even following EtOH ppt are due to the DNA. Despite removal of over half of the unincorporated dNTP only ~1.5% of the total counts are acid pptable
4. When EDTA was added & the [illegible] switched to Na acetate (in place of ammonium acetate) degradation of the DNA did not occur
5. Perhaps the Vitcs purified stuff—although very cold—is just pure DNA. The pellet was not very big, but counting it should tell for sure—after all EtOH ppt of

Ethanol ppt of the remaining raw rxn mix

#1 1072.5λ - 622.5λ = 453λ

#2 1072.5λ

#1

453λ of #1 (23 µg of DNA)
225λ of 200mM EDTA
45λ 3M Na Acetate

350λ of #1 (18µg of DNA
196.5λ of 200mM EDTA
39λ of 3M Na acetate
517.5λ EtOH
1225λ total

~~350λ of #1~~
~~3.5λ of 200mM EDTA~~
~~17.5λ 3M Na Acetate~~
~~350λ EtOH~~
~~717.5λ~~

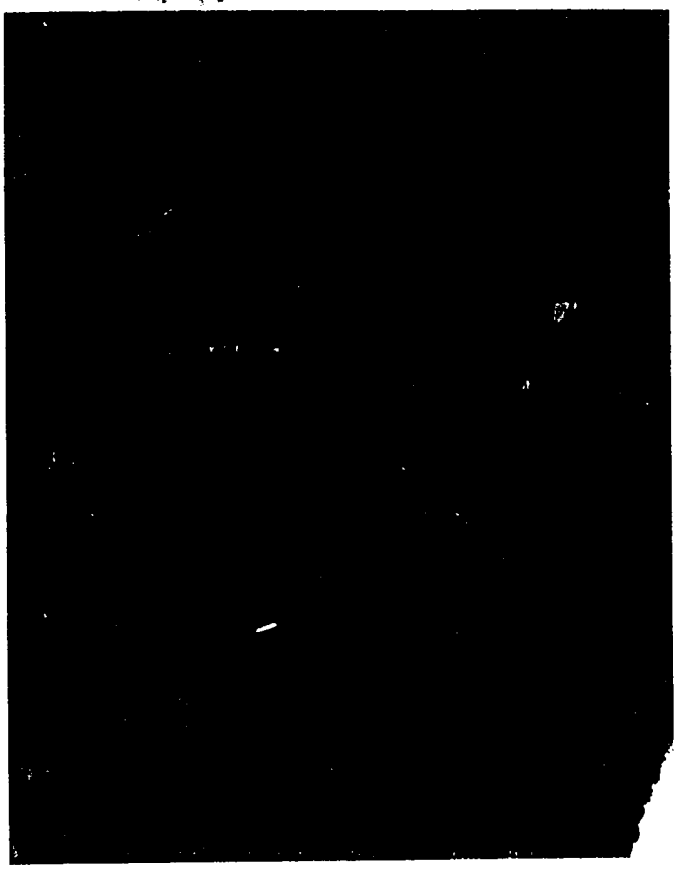
#2 — withdrawn to get reference & counting.
aliquots of 350λ and treat
as #1

#1 resuspended in 50λ TE
#2 resuspended in 50λ TE

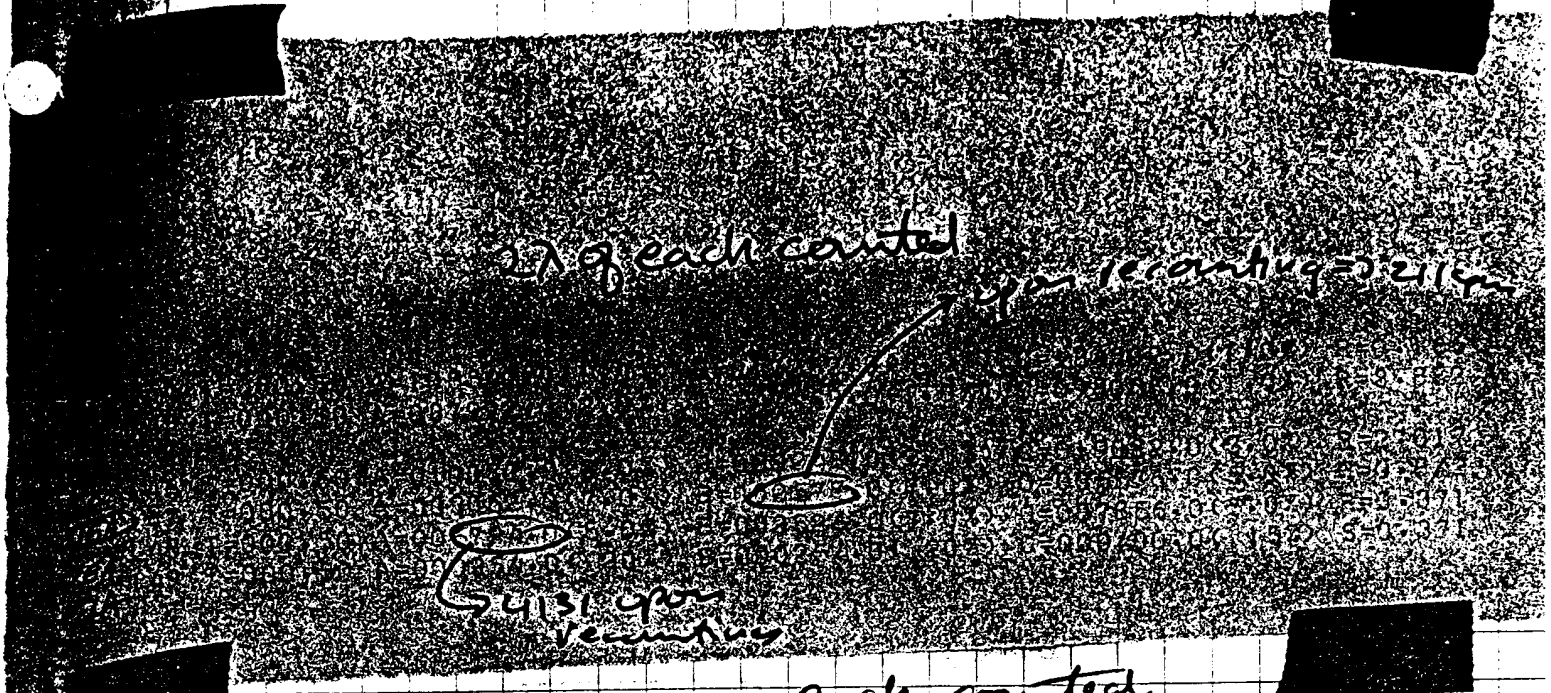
2λ withdrawn for counting

Both TEA ⟶ ppt as opposed to [illegible]
⟶ [illegible]

2 - [illegible] PM
2 - Ethanol ppt
1 - [illegible] ppt -
large bands



This gel shows that the DNA has maintained it's integrity following EtOH ppt.



I. Do these results jive w/ earlier pool & acid precipitable count data?

A. p.58W ⇒ check of "pool" results

^{3}H $\begin{cases} \frac{2}{5}(36{,}228) \stackrel{?}{=} 10{,}080 \\ 14{,}491 \not\approx 10{,}080 \end{cases}$

⇒ fairly consistent agreement

^{32}P $\begin{cases} \frac{2}{5}(76{,}976) \stackrel{?}{=} 20{,}348 \\ 30790 \not\approx 20{,}348 \end{cases}$

The ^{3}H data should be more accurate for this comparison because the ^{32}P is in continuous decay.

B. Check of acid ppt'able count results

^{3}H $\{ .2(1248) \stackrel{?}{=} 254$

$250 \approx 254$ ⇒ fairly consistent agreement

^{32}P $\{ .2(2008) \stackrel{?}{=} 254$

$402 \not\approx 254$

II, Recovery of EtOH ppt

A. Rxn #1

^3H { ~~1080(8000)~~ → 2λ 3052(1.5) / 1090(17.5) → 3λ ~~48% recovery~~ 24% recovery

1EA 350λ represents 350λ of 1A resuspended in only 20λ ⇒ $\frac{350}{20} = 17.5\times$ concentration upon recounting → 2λ

^{32}P { ~~2578(1.5)~~ / 66(17.5) = ~~96~~ 27% recovery ~~3.35% recovery~~

.25(18μg) / 20λ ≈ .23μg / 20λ ⇒ 4.5μg

Clearly, something has gone wrong as ^3H & ^{32}P don't jive

B. Rxn #2

4131 → upon recounting

^3H { 6994 / 254(20) = ~~34%~~ recovery 81% recovery

#2 was ~1000λ redissolved in 50λ ⇒ 20 fold dilution

^{32}P { 2526 / 254(20) = 50% recovery

⇒ on the average ~ ~~65% recovery~~ 65% recovery

⇒ .65(55μg) / 50λ = .72μg/λ

50λ = 36μg of DNA

III. Removal of dNTP

A. rxn #2

~~10,080 − 254~~
~~(37,992 − 6,794)~~

$^{3H}\left\{\frac{37,992-6,794}{(10,080-254)20}=.16\right.$ ⇒ 84% of all unincorp dNTP's removed

$^{32P}\left\{\frac{147,462-2,526}{(20,348-254)(20)}=.36\right.$ ⇒ 64% of all unincorp dNTP's removed

∴ ~75% of all unincorp. dNTP's remove

B. rxn #1

~~1090 − 66~~

$^{3H}\left\{\frac{(103,540-3052)(1.5)}{(44,566-1090)(17.5)}=.19\right.$ ⇒ ~~81%~~ of all unincorp dNTP's remo

$^{32P}\left\{\frac{(17,252-2578)(1.5)}{(10,928-66)(17.5)}=.12\right.$ ⇒ 88% of all unincorp dNTP's remov

Attempted Annealing, Fill & ligation

Anneal

2.8λ	#2 rxn/phenol'd/EtOH'd @ .72μg/λ	2μg (SC ↑ D ~ ↓ 20mM Tri)
.8λ	1M tris ph 7.5	
36.4λ	H_2O	
40λ	total	

1. heat to 68°C for 5 minutes
2. incubate at 58°C for 1½ hours

Fill-in & ligation

40λ	of #2 rxn/p'd/e'd DNA at 50ng/λ (from above) (in 20mM tris pH 7.5 (=2μg)	33.3ng/λ
12λ	of 54λ Klenow Pol I from BRL (1 unit) (=60 units)	1U/λ
1.2λ	.5M dTT	10mM
0.6λ	.1M ATP	1mM
0.6λ	1M $MgCl_2$	10mM
3λ	of 2U/λ T4 DNA ligase (BRL (=6 units)	.1U/λ
0.5λ	5mM dATP	42μM
0.5λ	5mM dCTP	42μM
0.5λ	5mM dGTP	42μM
0.5λ	5mM dTTP	42μM
59.4λ	total	

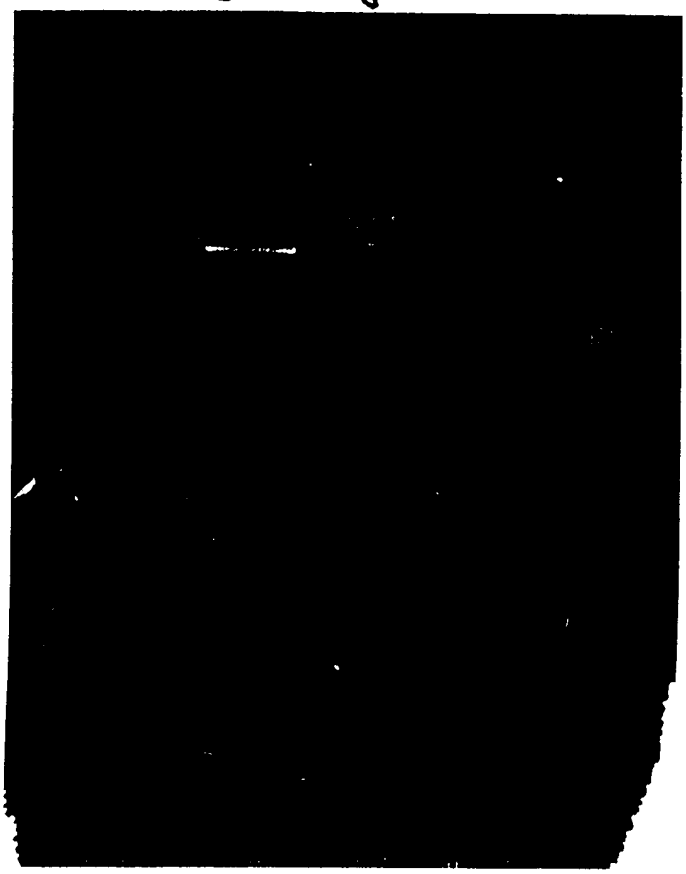
3:20pm Avg. 15
incubated at room temp = 22.2°C

(at room temp)

15' with [illegible] water cycle

scrung = 0.5 μg

ran 1/2 anneal mix



Is it circular or linear — try restricting it! yes, but restriction sites will randomly appear also assuming all went well

unique sites in pUC19 cont'g multiple cloning region → NAR ECORV HIND

~~Ligation mix (=267 ng DNA)~~

9:45 -- 1 ml of overnight → 200 ml x 4 T

The following plates were made

pUC19 (unmodified; uncut)	The pUC19/random
.5 ng	.5 ng (from "annealing mix")
5 ng	5 ng
50 ng	50 ng
	500 ng

The DNA was diluted accordingly

↳ incubated at 2:15 pm

Transformation Protocol

Ca^{++} Cells

1. Pick a single colony (or from stock glycerol) of JM83 and innoculate into a 20~~0~~ml 2xYT at 37°C in a shaker overnight. (Use a large flask ~~for~~ 250ml for good aeration

2. Take 1ml of this overnight culture and innoculate a 200ml 2xYT culture (a large flask again) for 2hr

   While growing place a 250ml sorvall bottle at -20°C to cool

3. At A_{600} = .6 or "just a little less" harvest culture by centrifugation at 5K for 8 minutes at 0-4°C.

4. Decant supernatant (clear and amber). Resuspend cell pellet in ice-cold 50mM $CaCl_2$ (filter sterile). Use 1/2 original grown volume (~100ml); resuspend by swirling; do not pipette to resuspend. Incubate on ice for 20 min. w/ occasional swirling

5. Repellet for 5K at 0-4°C for 8 min.

6. Decant supernatant (clear and colorless) and resuspend cells in 1/10 original volume (~20ml) of ice cold 50mM $CaCl_2$

7. Store cells on ice in cold room.

NB:
(i) keep everything cold! (on ice)

For amp selection: add
1 ml of 2XYT to each
tube and incubate in 37°C
water bath for 1 hour

transfection

1. For every desired plate, place 0.3ml Ca++ cells in a small culture tube and add DNA. Incubate 40 minutes on ice (20 minutes should be sufficient)

   Meanwhile, make top agar, 2ml per plate (2XYT heated in μwave with .9% agar; .09g/10ml don't let boil over in μwave; prepare in small conical culture flask) and keep warm on hot plate set at "4.2 and low" (=

2. After the 40 minutes is up heat shock the tubes for 2 min in a 42°C water bath (make this bath in a styrofoam tub w/ sink water, (warm to room temp the plates to be used)

3. Remove from heat and add to the soft agar the following on a per tube amount

   per tube, put add to soft agar & mix:

   15λ IPTG (.1M)
   75λ XGAL (=2, in DiMeFO (amide) 50
   6λ 37λ amp @ =5mg/ml => 75μg/ml)

4. Add 2ml of the above mix per each tube of Ca++ cells; quickly vortex and pour on to 8 agar AMP plates; swirl to coat the whole plate

5. Incubate upside down at 37° overnight

Results:

More or less a lawn on all plates ~~& control~~ even neg. control & AMP based

A few blue colonies on the periphery of the "19" plates "A-50" had 2 or 3 blue colonies on the periphery

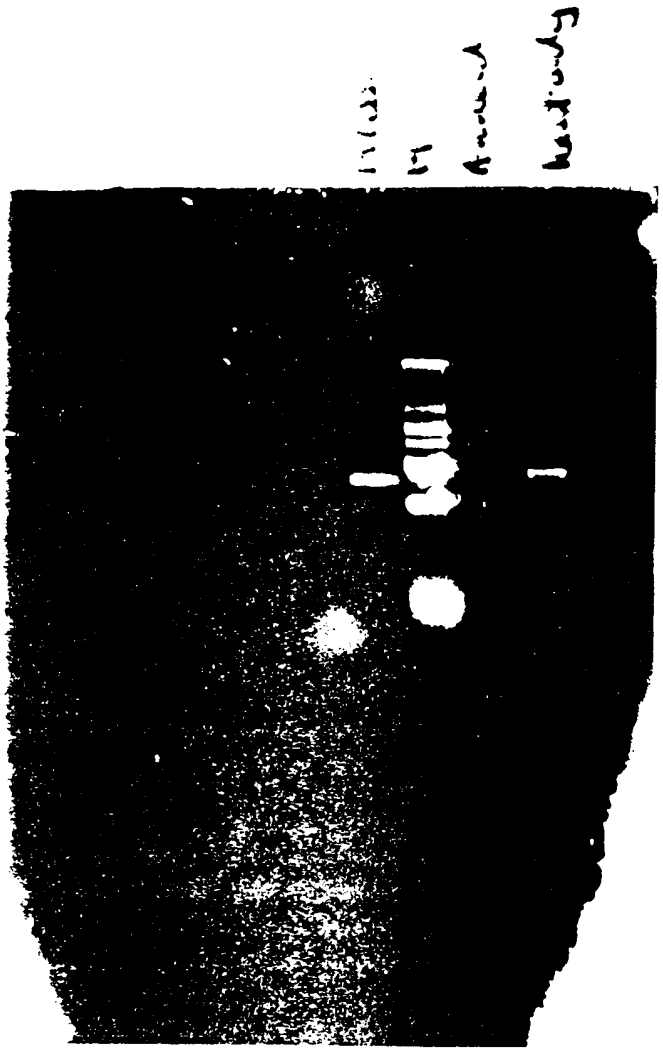
Making more "anneal II":

- 2x the protocol on p. 65 was followed → incubation at 65 for 5:50pm
- also "anneal control" at 1X the original protocol was made. It was heated only to see if the slower migration is merely an artifact of clumping under heat — NO! Known on ligure ladder — set out at room temp w/ "anneal II"

Rxn allowed to go O/N starting 7:15 pm → 8:30 am

↓

8 hour rxn



Transformation Data

Lb	[stock]	[plate]
Amp	25 mg/ml	50 μg/ml
tet	12.5 mg/ml	12.5 μg/ml
Strep	10 mg/ml	25 μg/ml
Chlor	34 mg/ml	10 μg/ml

→ The 2° Lb were not added to the top agar—only in the master plate

pUC19

DNA Dilutions

1 ng/plate ⇒ 3.8 λ q 1:100,000

5 ng/plate ⇒ 3.8 λ q 1:10,000

50 ng/plate ⇒ 38.5 λ q 1:10,000

[Stock] = 13 μg/λ

Anneal Mix

1 ng/plate ⇒ 1.5 λ q 1:100

5 ng/plate ⇒ 15 λ q 1:100

50 ng/plate ⇒ 1.5 λ q, undil

500 ng/plate ⇒ 15 λ q undil

Media

B broth: 10 g bacto tryptone
8 g NaCl
add 1 liter dH_2O & autoclave (liquid)

B plates: Add 15 g/λ Agar for plates

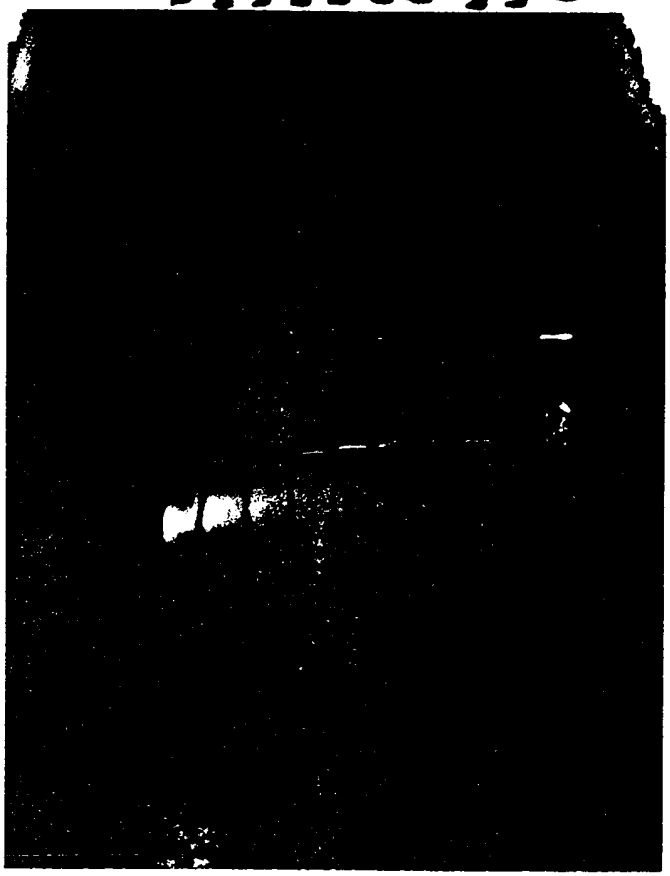
Nomenclature for Replica Plating

W ⇒ white colony picked from PUCRAN plates
WB ⇒ blue colony " " " "
B ⇒ blue colony picked from PUC19 plates

* The number following the above designation is arbitrarily assigned to the colony p

W☆ } ⇒ These colonies were picked from the plat
B☆ } according to the above Nomenclature (minus the ☆), but they were NOT replica-plated. Because I worried for the plasmid prep that my liquid tube would not grow I picked a couple of colonies that would not "be replaced on the loop" by replica plating and also used to inoculate liquid culture (for the plasmid prep) only.

Rx Plasmid prep from Transfected cells 070



The DNA was eluted in 20λ

4λ was run on the gel

070

5ml total

1 ml 5M NaOH
250 µl 20% SDS
3.75 ml H_2O
5ml

40ml

.32g
4ml 10%
30ml H_2O

200 ml Sol'n II

.16g NaOH
1ml 20% SDS
19ml H_2O
20ml total

for 150ml 1.2g NaOH .20g
15ml 7.5ml 20% SDS 1.25ml 20% SDS
135 142.5 23.75ml
150mls 25ml

10% SDS
240ml
1.92g
8ml
160 216
72mls H_2O
80

80mls
.64g
8mls

Quick Prep
Modified from
Birnboim & Doly (1979)
NAR 7, 1513-1523

Quick plasmid (cosmid) preps.

Soln I = 50 mM Glucose
25 mM Tris pH 8
10 mM EDTA (pH 7.5)
~~10~~ 18 mg/ml lysozyme (fresh) (for cosmids ~~make 10 mg/ml~~). see over

Soln II = 0.2 N NaOH
1% SDS (~~keep 1 week~~ make fresh!!!)
1.25 ml 10% SDS
11.25 ml H_2O
0.1 g NaOH = 1 pellet

Soln III = 5 M KOAC pH 4.8 (60 ml 5M KOAC + 11.5 ml HOAC → 100 ml).

5 ml total:
2.5 ml 0.2 N NaOH
0.5 ml 10% SDS
2 ml H_2O

5 ml total:
250 µl 20% SDS
100 µl 10N NaOH
4.65 ml H_2O

1. Spin down 1 ml of o/n culture in Epptube 15 secs.
Remove all medium w. 1 ml Gilson. (If treating large nos. of samples, can use with drawn out pasteur pipette on a water-pump. Ditto for rest of protocol).
See over for 5 ml. cosmid cultures).

→ 50λ w/o lysozyme
then add 50λ w/ 2x lysozyme

2. Suspend pellet in 100 µl Soln I with Eppendorf & vortexing.
(for cosmids, transfer to Eppendorf tube)
5 min at R. Temp.

3. Add 200 µl Soln II with Eppendorf. Mix by rocking tube sharply but don't vortex.
Inc. on ice for 5 min.

4. Add 150 µl precooled Solu III. Seal cap well, invert tube, mix gently on lid, vortex gently briefly, invert tube (to normal orientation); vortex gently briefly.

5. Inc. on ice/5 min.

6. Spin 1 min/microfuge.

7. Transfer supernatant (360 µl) to fresh tube, avoiding sediment.

~~7a add 1/2 DEP → incubate at 65° for 10-15 min~~ ?

8. Add 720 µl EtoH
Leave 2 min at R. temp.

9. Spin 1 min/microfuge.

10. Remove supernatant, carefully but completely with 1 ml Gilson.

11. Wash with 500 µl 70% EtOH, rinsing sides of tube with inclining tube (no vortexing or spinning)
Remove carefully but completely with 1 ml Gilson.

Dry (2 min/high vacuum).

Take up in 20 ~~50~~ µl TE, suspending pellet w Eppendorf.
Vortex, 4°/RT, vortex.

Before removing aliquots for digestion (~ 2 µl) (~~5 µl for cosmids~~) 4λ
Clarify by spinning 10 sec/microfuge.

~~Add RNase in digests.~~ ~~Can be scaled up preparatively~~ ~~used Qiagen~~ ~~prep~~.

PHENOL EXTRACT

PRECIP

Restriction Digests of QUICK plasmid preps

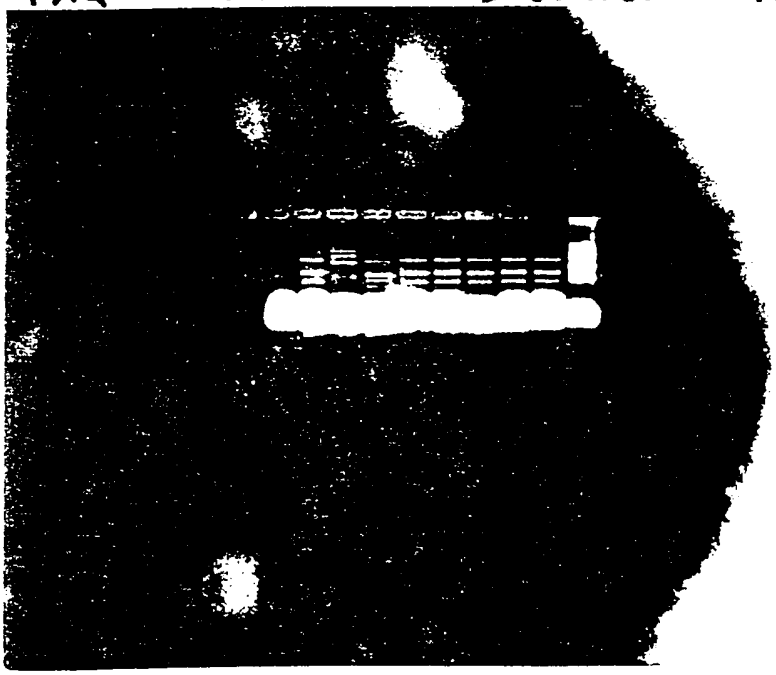
3λ of plasmid prep DNA
1.5λ of Taq I Restriction Endonuclease @ 10 u/λ
1λ of 10X core buffer
4.5λ of H_2O

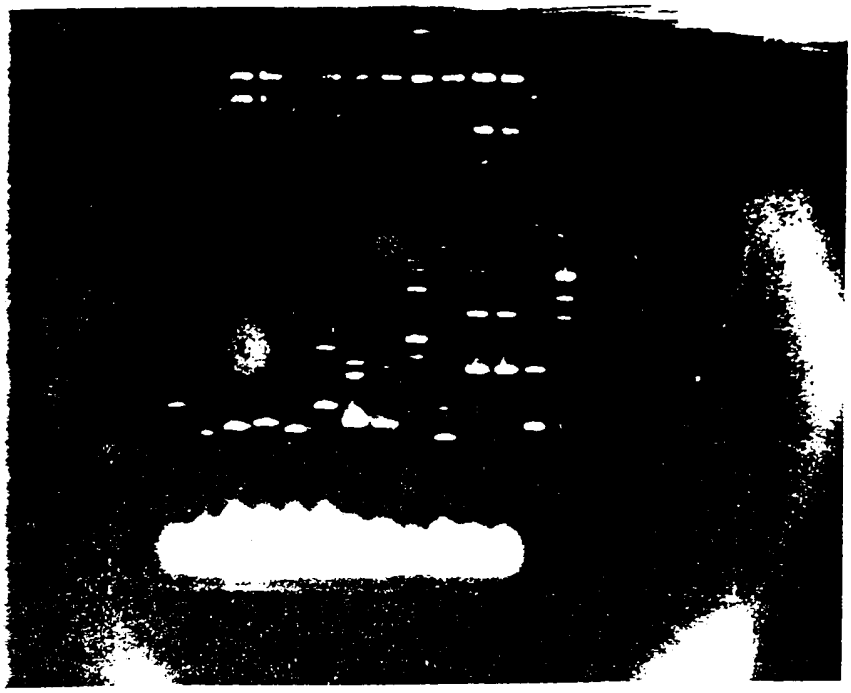
incubate at 37°C

+ 1μ stock digest
.3λ PUC19
1.5λ of Taq I
1λ of 10X core buffer
7.2λ of H_2O

· 60min at 37°C

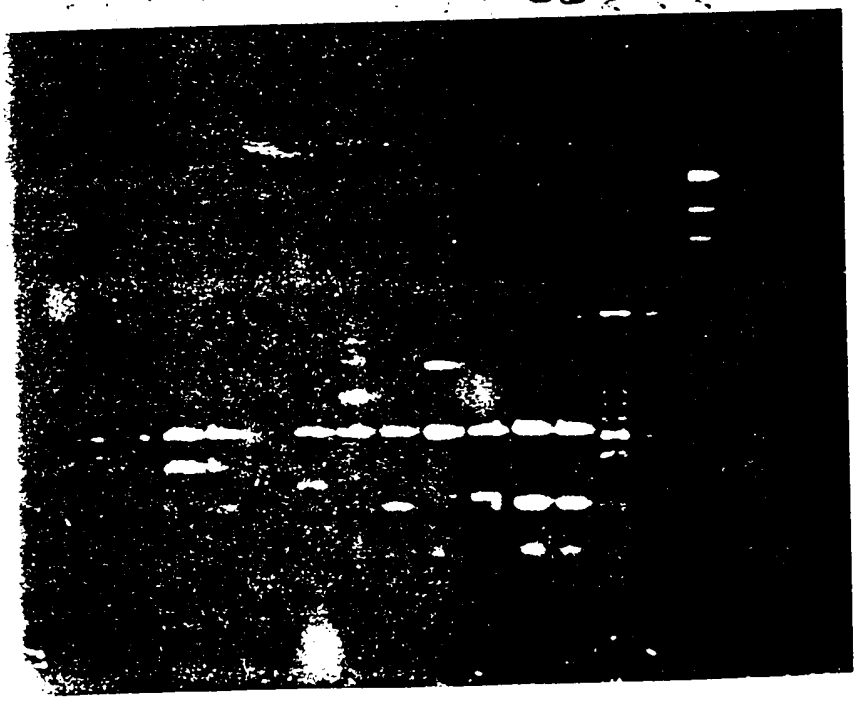
TAQ Digest W1·W2·W3·W5·B1·B2·W81·W82·19

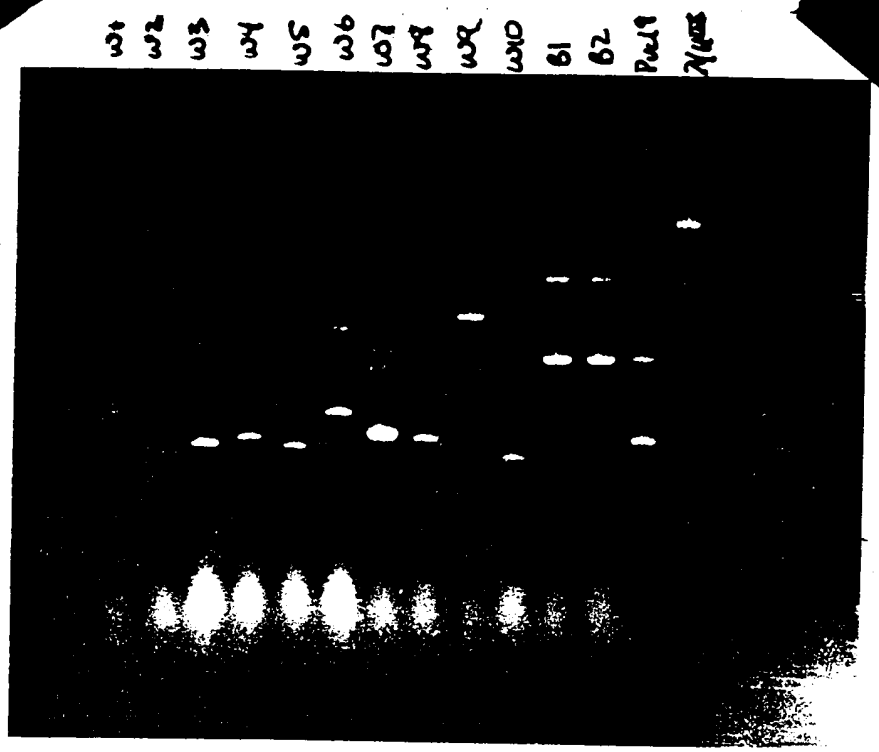




CUT

CUT

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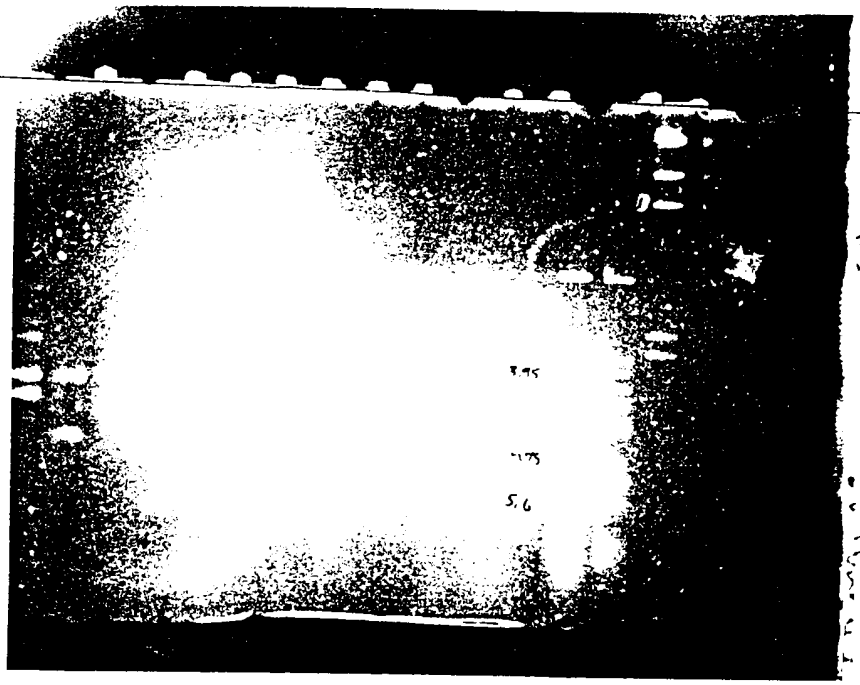
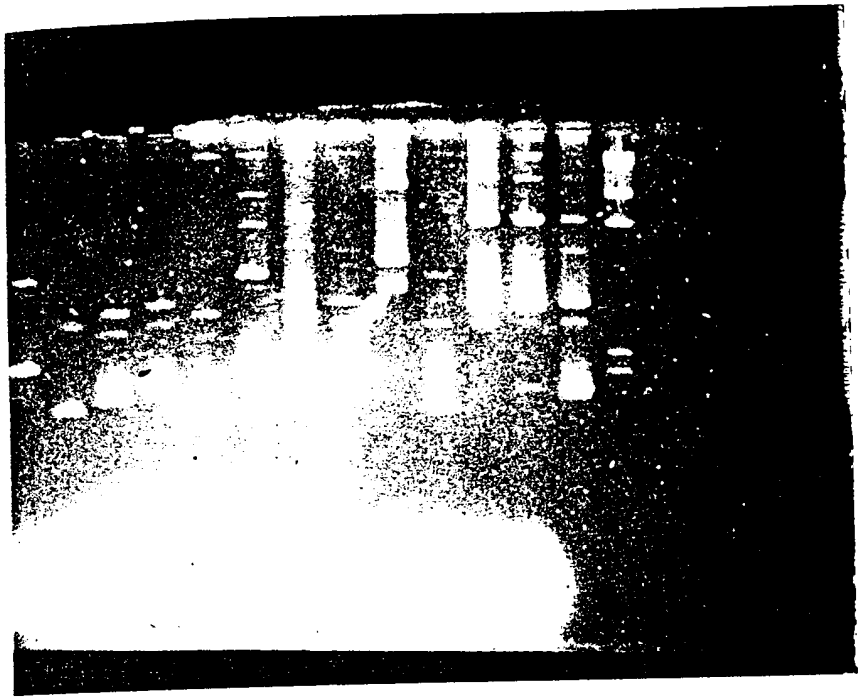
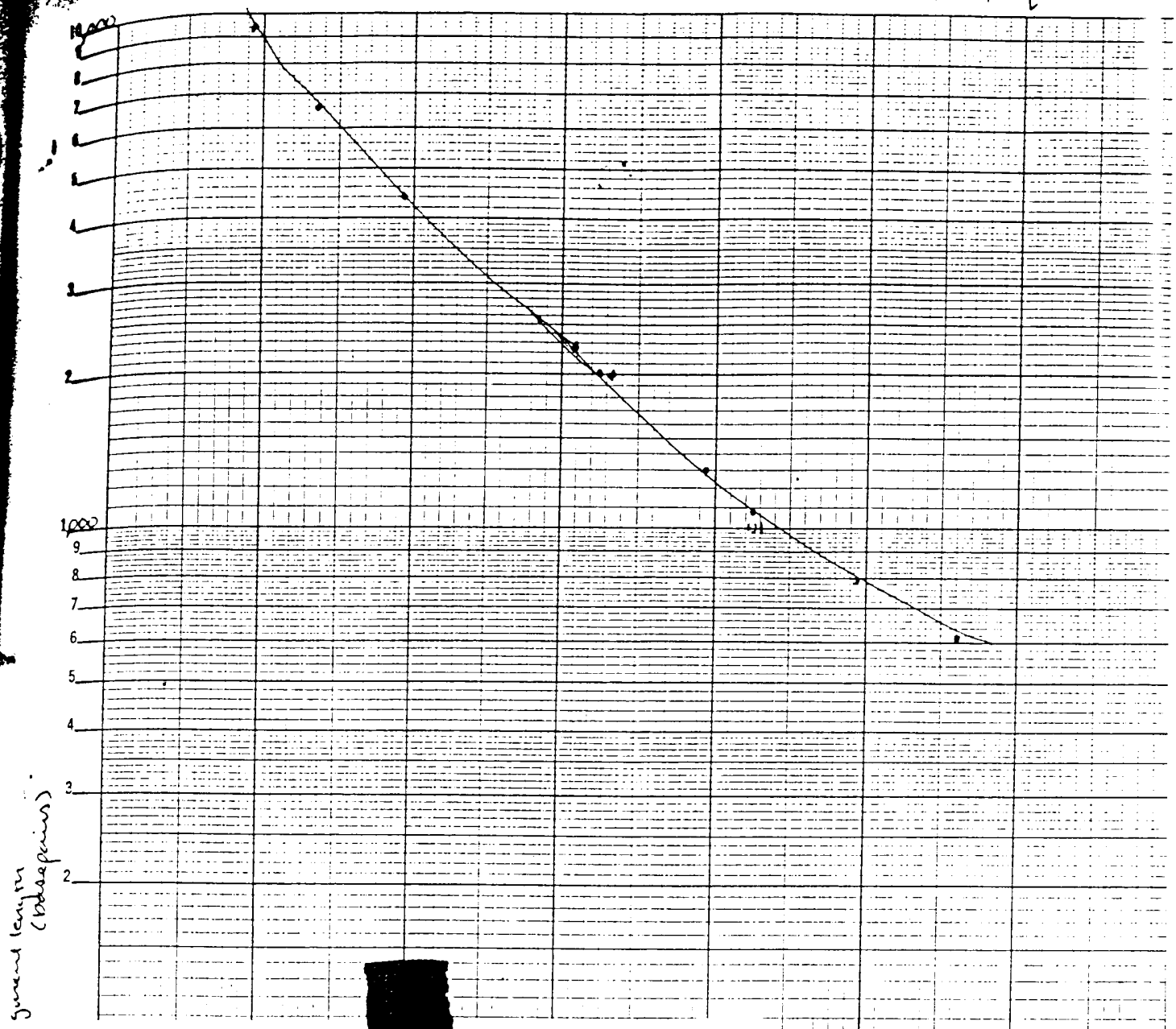
Pwl9
λ/MspI
UNCUT


CUT
5.6



Uncut

Standards from λ/H.ind III
PUC 19/Taq I



[redacted]

W1	W2	W3	W4	W5	W6	W9
[illegible]	1.3Kb	1.3	1.3	ϕ	1.9	2.2Kb
	.85	1	.8		.6	1.3Kb
2.5Kb			.6			.5Kb
2.0Kb						
1.3 Kb						
1.1 Kb)						

(start & fast calculation of size of bands)

[redacted]

: growth for 15 hrs (in cover, under [illegible]
tried:

fox-5	1.98 l/ml	1.9 λ stock
fox-6	8.5 8 l/ml	.9 λ stock

	sulf	trimeth	
ST-5	5 8 l/ml	1.8 l/ml	35 λ of 1:10 stc
ST-6	2.5 8 l/ml	.5 8 l/ml	1.8 λ of 1:10 stc

FOX4-A has growth (but FOX3-A does not, while all other foxes do) except for FOX4-19 & FOX3-19. (From 9-25 → 9-27 these were allowed to sit at room temp.)

3 (1 ML) of FOX4-A was withdrawn & pelleted; S.N decanted & pellet frozen at -70°C (Stored in Ray's "In Use" box) to save for future plasmid prep.

100 ml of FOX4-A was dumped onto a ZKYT plate and dB sensitivity discs applied.

From the 2° plates (STX & FOX) all colonies were lifted (one at a time w/ a loop) and added to 2.5 ml liquid ZKYT, shook overnight at 4°C, and then 100 ml of each was dumped onto a ZKYT plate

Selection Experiment

Goal: To see if any of the pUCRAN DNA will confer drug resistance

Methods:

JM83 was transformed with pUCRANIIDNA (≡ "AII") @ 300 ng per plate (total of 2 plates) according to the protocol on p. 66y (Control: pUC19 @ .5 ng) (AII → ~75% white colonies; pUC19 ~100% blues)

"lifts" → On 6 ml of 2xYT liquid was added to plate - swished and added and raked (w/ glass rake). 200 λ of this infective liquid was plated onto large diam. petri AMP, 2xYT plates (AMP @ 50 λ/ml). Antibiotic disks were added to the plates (~30 different ones). There were a total of 4 plates: 2 "lifts" from pUC19 controls (in ¶ above) and 1 each from the two AII (≡ pUCRANII) DNA. (The II merely denotes the second batch prepared according to the protocol on p. 65w).

diameter of

		Pvc19#1	Pvc19#2	ATT#1	ATT=
nofurantoin	F/M	2cm	2.2cm	2cm	
sulfamethazol	TH	Ø	Ø	Ø	
sulfamethazide trimethoprim	SXT	1.1cm	1.6cm	Ø	
ampicillin	AM	Ø	Ø	Ø	
tracycline	TE	2cm	2.3cm	2.1	2
polymixin B	PB	1.2cm	1.2cm	1.2	1.2
	NN	1cm	1.1cm	1.1	1.1
cefuradol	MA	Ø	Ø	Ø	Ø
	CF	Ø	Ø	Ø	Ø
ticarcillin	TIC	Ø	Ø	Ø	Ø
gentamicin	GM	1.2cm	1.3	1.1	1.2
moxalactam	MOX	2.7cm	2.7	2.9	2.5
	FOX	2.4cm	2.4	2.4	2.4/1cm gram
cefotaxime	CTX	2.5cm	2.6	2.7	2.6
pipericillin	PIP	Ø	Ø	Ø	Ø
cefoperazone	CFP	Ø	Ø	Ø	Ø
	AN	1.5cm	1.5	1.4	1.4
chloramphenicol	C	2.7cm	2.7cm	2.4	2.4/1cm

sulfamethoxazole trimethoprim = SXT
cefoxitin = fox 30

ST-A colonies picked from single colonies growing around SxT discs on 2° plates

ST-19 colonies
FOX-19 colonies } picked from lawns on big 19-plates

FOX-+ colonies picked from single colonies on 2° plates grown in absence of ~~FOX~~ (but incubated first)

Liquid broth

Cefoxitin stock

50 mg/ml
= .05g x 10ml = .5g in 10ml

Each tube: 5ml 2xgT
10λ of 25mg/ml AMP ⇒ 50δ/ml

Cefoxitin (AMP is labelled 10 MCG from disk package; FOX is called 30 mcg)

fox-1	37.5δ/ml	3.8λ of stock
fox-2	75δ/ml	7.5 λ stock
fox-3	150δ/ml	15λ of stock
fox-4	300δ/ml	30λ of stock

Sulfamethoxazole trimethoprim

stock: 16mg/ml trimeth
80mg/ml sulfa

	Sulf	Trimeth	
ST-1	10δ/ml	2δ/ml	.7λ of stock
ST-2	20δ/ml	4δ/ml	1.3λ of stock
ST-3	40δ/ml	8δ/ml	2.5λ of stock
ST-4	80δ/ml	16δ/ml	5λ of stock

for 4b disc sensitivity testing.

In going through old plates a single colony was noticed on a month old TET plate, it was picked & grown 2 days in air shaker at 37°C (in 12.5 µl/ml 6.3 µl/ml, 3.2 µl/ml, and 1.6 µl/ml [TET] against controls)

For all pUC19 controls, a loop was dipped into an area of the lawn on the very large, 1° 4b sensitivity plates (already from the discs)

liquid
~~Colonies~~ picked for plasmid preps

TET @ 1.6 µl/ml + 50 µl/ml AMP — 2 days 37
2 days R

19 @ 50 µl/ml AMP
+ 5 µl/ml SULF
1 µl/ml trimeth — 2 days 37°C
2 days RT — TET from plate

SXT @ 50 µl/ml AMP
+ 5 µl/ml SULF
1 µl/ml trimeth — 2 days 37°C
2 day RT — Selected 2° plate

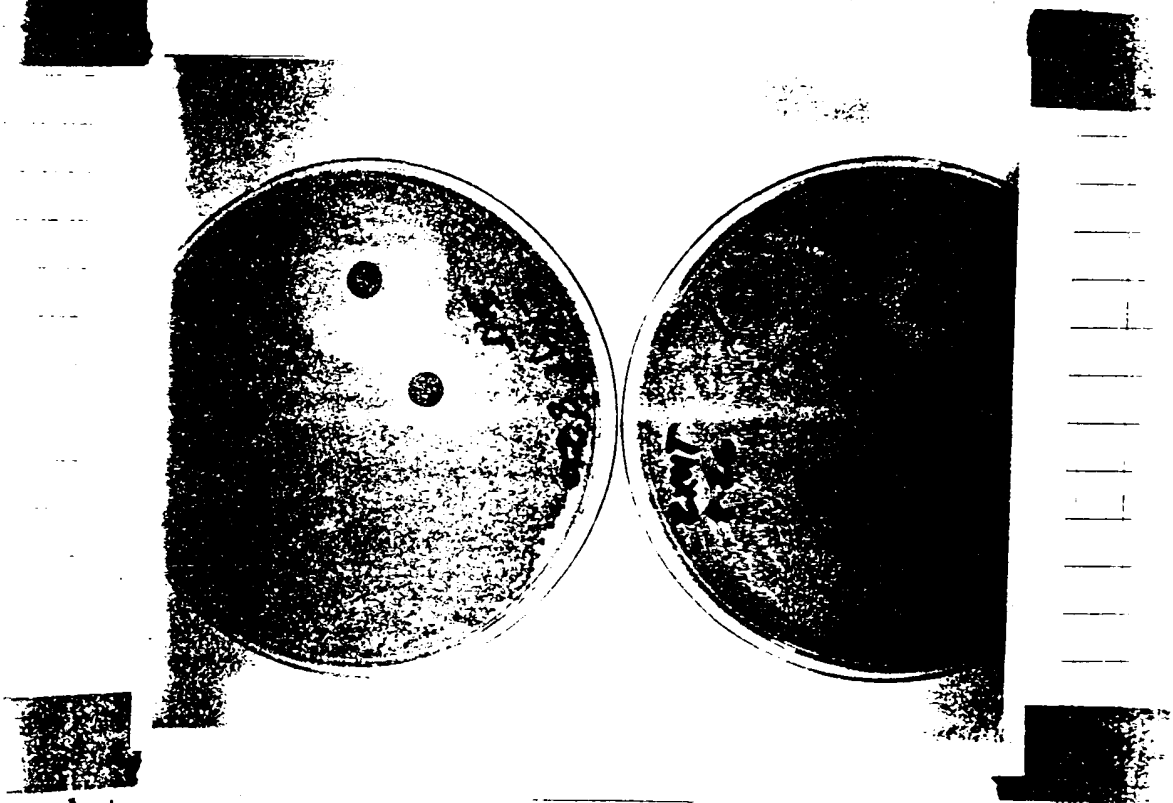
SXT @ 50 µl/ml AMP
80 µl/ml SULF
16 µl/ml trimeth — 2 days 37°C
2 days RT — Selected 2° plate

FOX @ 50 µl/ml AMP
250 µl/ml cefoxitin — 2 days 37°C
2 days RT — unselected 2° plate

Pursuit of SXT resistance

Colony isolation

The SXT^{res} colonies from the original screening were plated onto 2° amp/kan (12 grid plate. All colonies from this 2° grid (except edge) were picked and inoculated into 2ml 2XL liquid. 100 λ of this inoculum was spun onto a 2XYT plate and checked for AMP & SXT sensitivity w/ the ds paper discs. A pattern similar to the original screening was observed. (The photo below is a picture of the second screening plate)



A loop taken from as close to the paper disc as possible was smeared onto an LAMP plate to isolate single colonies. Single colonies were isolated and grown on an LAMP grid plate in which the agar pricks were made around the edge of an SXT ds paper discs. These are then isolated single SXT^{res} AMP^{res} colonies (PUC19 prepared as above - colonies picked from the lawn on the original selective plate - did not grow at all near the SXT discs).

← see p. 74

The above single colonies were used in the following liquid inoculum:

Each tube: 5ml 2XYT
50 µl/ml AMP

	sulf	trimeth
ST-4	~~80~~ 80 µl/ml	16 µl/ml
ST-3	40	8
ST-2	20	4
ST-1	10	2
ST-0	⊖	⊖

5 λ q stock
2.5 λ q stock
1.3 λ q stock
.7 λ q stock
⊖

The following the label an "A" denotes putative STEs and an "19" indicates pUC19 control.

The colonies were inoculated into the 2XYT+A and allowed to grow for 1 hour 15 minutes before the sXT was added.

pUC19 controls are from single colonies pUC19's on an AMP plate

results

	+	19
ST-4	⊖	⊖
ST-3	+	+
ST-2	+	+
ST-1	⊖	+
ST-0	+	+

⊖ = NO
+ = Grow

ST-4 A & ST-019 grown for plasmid preps

preps digested!

3λg DNA
1.5λg Taq I
2λg
2.5λg
10λ tot

65c

8:35am

19/Taq
S8/Taq
SH1/Taq
SH2/Taq
Lwls
SH1
SH2